ISOZYME STUDIES IN *ANTHURIUM ANDRAEANUM* AND RELATED SPECIES

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ABSTRACT

Studies were conducted to assess the feasibility of the use of gel electrophoresis of different isozyme systems as a tool to examine the genus Anthurium. Procedures were also developed to extract active enzymes from anthurium tissues.

Clear zymograms were obtained from only one of three extraction methods examined. This method consisted of grinding the tissue in liquid nitrogen and then adding a buffer containing reducing agents, a phenol oxidase inhibitor and PVPP to the ground tissue.

Peroxidase tissue specificity was assessed using three A. andraeanum cultivars. Some specificity among tissues within each cultivar was noted. However, similarities and differences of the banding patterns among the three cultivars were not consistent.

The identification of seven A. andraeanum cultivars was undertaken using seven enzymes systems. Banding was noted in four of these enzyme systems; glutamate-oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), peroxidase (Px) and phosphoglucose isomerase (PGI). Characterization of all seven cultivars was indicated by the combined data of Px, MDH and PGI.

Thirty six species were used to evaluate the significance of peroxidase banding patterns in anthurium taxonomy. A total of 32 peroxidase band positions were recognized for the species surveyed. UPGMA cluster analysis was applied to the data. The analysis indicates that isozymes in Anthurium have some taxonomic value. Additional work is required, however, before practical use can be made of the technique.

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I. INTRODUCTION

The genus Anthurium is comprised of over 700 species (Sheffer and Croat, 1983). A. andraeanum is an important cut flower crop in Hawaii, while A. scherzerianum is especially popular in Europe, grown as a pot plant. Other species such as A. warocqueanum, A. crystallinum, A. hookeri, A. trinerve and A. scandens are grown as foliage plants.

In the anthurium breeding program at the University of Hawaii, extensive intraspecific hybridization as well as interspecific hybridization has been carried out to improve flower quality and create new forms. The ease with which A. andraeanum hybridizes (Sheffer and Kamemoto, 1976b; 1977) and the meiotic irregularities in pollen mother cells found in a few cultivars (Kaneko and Kamemoto, 1978) suggest that A. andraeanum is of hybrid origin. Birdsey (1956) noted that the spathe of A. andraeanum as described by Linden was orange-scarlet in color and blistered in texture, whereas cultivars found commercially range from white to dark red and vary in texture. The name A. x cultorum Birdsey has been proposed to express the hybrid character of these plants. To aid in the understanding of this complex genus, cytological and biochemical evaluations of hybrids and their parents have been and are presently being conducted (Kaneko and Kamemoto, 1978; Sheffer, 1974;

Iwata 1980). Thus far, biochemical evaluations of anthuriums have not included the use of isozymes.

The study of isozymes in anthurium may be useful in elucidating species relationships and help clarify the hybrid origin of the present day A. andraeanum cultivars. Isozyme banding patterns can be used as "finger prints" to cultivars and aid visual identification of taxa (Peirce and Brewbaker, 1973).

The purpose of this study is to examine isozyme polymorphisms, their use as taxonomic aids and in cultivar identification in Anthurium.

II. LITERATURE REVIEW

2.1 Anthurium

Anthurium Schott is the largest genus in the family Araceae consisting of over 700 species (Sheffer and Croat, 1983), with a distribution ranging from central South America to Mexico and the West Indies (Sheffer and Kamemoto, 1976a). This genus can be identified by a persistent spathe, spreading or reflexed, sometimes showy, spadix densely covered with bisexual flowers, petioles geniculate, stems short or elongate and leaves usually firm with primary lateral veins mostly connected by a well defined vein running along inside and paralleled to margin, smaller veins always reticulate (Bailey Hortorium, 1976). A classification was constructed by Engler (1905) in which he divided the genus Anthurium into sections. Categorizing all 486 species known at the time into 18 sections, Engler based his classification primarily on the number of ovules per locule, leaf shape and texture, infloresence shape and berry shape. Sheffer (1974) found Engler's sections often to be ambiguous for assessing species relationships and therefore, constructed a new classification of six morphologically related groups. The key to these groupings were based on characters similar to those used by Engler (Sheffer and Kamemoto, 1976b). Recently however, Engler's sections have been greatly

modified and augmented (Sheffer and Croat, 1983). Croat and Sheffer (1983) have constructed a new key for identifying sections based primarily on leaf shape and venation, stem size and shape, and number of ovules per locule.

2.1.1 Inheritance Studies in *Anthurium*

To date, there has been little work done on the genetics of anthuriums. The few studies that have been conducted, have dealt mainly with the inheritance of spathe color. Kamemoto and Nakasone (1955) first studied the genetics of spathe color in the species *A. andraeanum*. They reported white spathed plants to be true breeding. White crossed with red produced all pink offspring. Pink crossed to white produced a 1:1, pink to white segregation ratio. Kamemoto and Nakasone (1963) further studied color inheritance by crossing white, red and orange spathed plants. Red crossed with red produced all red progeny. Crosses of orange x red produced either all red progeny of a 1:1 segregation ratio of orange to red. Orange selfed produced a 3:1 ratio of red to orange. The data from these studies suggested that spathe color was controlled by a multiple allelic system in which red was dominant to both orange and white.

Studies on interspecific hybridization of *A. andraeanum* and closely related species (Sheffer and Kamemoto, 1977)

revealed that spathe color inheritance is controlled by at least two pairs of genes. Although the data were not detailed enough to determine the exact mode of inheritance, complementary gene action was indicated. The inheritance of spadix color was found to be similar to that of spathe color.

A more recent study on spathe color in A. andraeanum involved qualitative and quantitative pigment analyses. Based on both visual observation and biochemical analysis, Iwata et al. (1979) proposed that each of the two major anthocyanins, cyanidin 3-rhamnosylglucoside and pelargonidin 3-rhamnosylglucoside, was monogenically controlled. Iwata (1980) concluded that an incomplete dominance form of interallelic interaction involving dosage effects on the concentration of each pigment was involved. Evidence of recessive epistasis was also observed.

The inheritance of B chromosomes in some Anthurium crosses have also been studied. Sheffer (1974) reported chromosomal inheritance of B chromosomes in 250 hybrids to be irregular. The data indicated that the effective inheritance of B chromosomes to be non-random and no evidence of B chromosome accumulation was observed. Kaneko and Kamemoto (1979) studied the B chromosomes of A. warocqueanum. The A. warocqueanum plant was determined to be 2n = 30 + 3B by Sheffer and Kamemoto (1976a). Kaneko and Kamemoto (1979) proposed that if the three B

chromosomes were equally functional in both the pollen and egg cells and were distributed at random, all seven types from 0 to 6 B chromosomes at the ratio of 1:6:15:20:15:6:1 can be expected in a large population. All seven types of B chromosomes were found in a study involving 94 selfed progeny of the A. warocqueanum plant with 2n = 30 + 3B (Marutani and Kamemoto, 1983). Plants with 3B chromosomes are expected, from random distribution, to occur in the highest frequency. The data however, showed plants with 2B chromosomes occurred with the highest frequency. Marutani and Kamemoto suggested the lagging tendency of univalent B chromosomes to explain this occurrence. The number of B chromosomes did not have a effect on morphology of the plant. However, B chromosome were found to have a slight effect on A chromosomes. Plants with more B chromosomes exhibited a wider range of A chromosomal configurations.

2.2 Gel Electrophoresis of Isozymes

In recent years, a number of plant species have been studied by isozymic analysis by gel electrophoresis. Various applications of this analysis include phylogeny, cultivar identification, tissue differentiation, and identification of genetic markers (Peirce and Brewbaker, 1973; Markert, 1977).

The use of isozyme analysis has become popular for a number of reasons. One strong advantage to the gel electrophoresis technique is its simplicity. All that is needed is an electric gradient and a medium for the protein molecules to move in. Also, many tissues can be assayed in a short period of time. The results are rapid and provide an accurate cultivar labelling system (Bringhurst et al., 1981).

2.2.1 Cultivar Identification by Isozymes

Aside from visual identification of taxa, isozymes provide a supplemental method of identification. Isozyme banding patterns or zymograms can provide good records of cultivars (Kuhns and Fretz, 1978b).

Bringhurst et al. (1981) characterized strawberry cultivars by studying the isozyme patterns of three enzyme systems - phosphoglucose isomerase (PGI), leucine amino peptidase (LAP) and phosphoglucomutase (PGM). Among the cultivars analyzed, PGI exhibited six different banding patterns, LAP had four different patterns and PGM displayed five different patterns. Combining the data of any two enzyme systems greatly improved the discrimination of the cultivars over any one system. The use of all three enzyme systems provided still greater discriminating power. The banding patterns of just these three systems identified 14 of the 22 cultivars analyzed. Similar studies on cultivar

identification by gel electrophoresis has been demonstrated in many plant materials such as grape (Schwennesen et al., 1982), pecan (Mielke and Wolfe, 1982), olive (Pontikis et al., 1980) and some floricultural crops.

Eighteen poinsettia cultivars were studied with the enzyme system peroxidase (Werner and Sink, 1977). After staining, four distinct banding patterns were observed for the 18 cultivars. One cultivar 'Truly Pink', could be identified based on its peroxidase zymograms. Kuhns and Fretz (1978b) found that eight rose cultivars could be divided into four natural groupings by the combined use of two systems, esterase and polyphenoloxidase. Using two other systems, cytochrome oxidase and phenoloxidase, all eight cultivars could be distinguished from each other. In studies of Camellia japonica, Wendel and Parks (1983) distinguished allozyme variation at 15 loci from 12 enzyme systems. This led to the unique classification of 165 out of 173 clones. This unparalleled study illustrates the astounding sensitivity available through the study of isozymes.

2.2.2 Isozymes as Genetic Markers

In the past, the most commonly used markers in evolutionary, taxonomic and breeding studies were morphological and cytological traits. More recently, with the increase in the efficiency and resolving power of

protein electrophoresis, biochemical traits such as isozymes are also used as markers. Isozymes provide a naturally "built in" marker system for investigations in biochemistry, developmental biology as well as the genetics of plants (Scandalios, 1969). They make ideal markers as they are colinear with the gene, commonly co-dominant in effect and are also relatively unaffected by the environment. Before isozymes can be used as markers however, an understanding of the inheritance in isozymes is necessary.

Maize has long been a favored crop for genetic studies. The vast genetic and cytological information available predisposes it for the study of isozyme inheritance. To date several polymorphic enzyme systems were reported to be controlled by over 30 loci (Scandalios, 1969; MacDonald and Brewbaker, 1974; Nagai, 1981).

The inheritance of polymorphic enzyme systems in recent years has been studied in diverse plant materials. Guries and Ledig (1978) proposed that eight enzyme systems were controlled by 14 loci in pitch pine. In citrus, four genes with 19 co-dominant alleles were found to control three enzyme systems (Torres et al., 1978) . Five polymorphic enzyme systems of date palm are believed to be encoded by seven genes having 14 alleles (Torres and Tisserat, 1980). Six enzyme systems found in the leaf and fruit tissues of avocado were found to be specified by 12 genes having 37

co-dominant alleles (Torres and Bergh, 1980). The use of isozymes has a definite advantage when used on long-lived and/or slow growing perennials, because this method allows the use of young seedlings, reducing the amount of time, space and other resources normally needed in genetic studies.

2.2.3 Tissue Specificity of Isozymes

The total enzyme repertory of any plant material changes during development and differentiation, thus tissue specificity of isozymes is not uncommon (Scandalios, 1969). In maize, only a few esterases were detectable in all of the 14 tissues examined by MacDonald and Brewbaker (1975). Several isozymes however are characterized by only a single tissue. For example, isoesterases of locus E4 were found to be root specific, while E10 isoesterases showed pollen specificity. Esterase pattern changes of developing fertile anthers were noted by Abbott et al. (1984). Pattern changes were observed as the pollen mother cells proceeded through the first mitotic division. Although different from those observed in fertile anthers, anthers from male sterile plants with cms S cytoplasm also exhibited pattern changes. Plants with cms C or cms T cytoplasm however, did not display any esterase pattern changes.

The peroxidases in maize also show great tissue specificity. Seven basic groups of maize tissues were distinguished by isozyme patterns. Of the 13 peroxidase isozymes found, none were present in all tissues examined (Brewbaker and Hasegawa, 1975). Tissue specific peroxidases have also been reported in tobacco (Bassiri and Carlson, 1979) and in common bean (Bassiri and Carlson, 1978).

III. MATERIALS AND METHODS

3.1 Plant Material

Anthurium species and cultivars used in this study were grown under saran at the mauka Manoa campus of the University of Hawaii. Many of the species and cultivars have been collected from Central American and Caribbean countries or from local private and commercial growers.

A total of seven cultivars were analyzed, four of which were cultivars developed and released by the University of Hawaii. 'Uniwai' arose from a cross between 'Haga', an accession with a white spathe, and Accession 12, with a creamish white obake spathe. 'Uniwai's spathe is broad, fairly smooth and heart-shaped with overlapping lobes. The spadix is yellow and reclining when young but turns upright with maturity. 'Marian Seefurth' is a selection from a cross between 'Haga' and Accession 11, a dark pink obake. It has a large, broad, heart-shaped, pink spathe with overlapping lobes that are fused at the base. The spadix is greenish-yellow and similar to 'Uniwai' in that the spadix is reclining when young and upright when mature. The cultivar 'Manoa Mist' is the result of a cross between 'Uniwai' and 'Marian Seefurth'. It has a fairly large white spathe with overlapping lobes and a reclining yellow

spadix. The pink cultivar 'Paradise Pink' is a recent University of Hawaii release. It originated from a cross between 'Marian Seefurth' and 'DeWeese', a white cultivar. Its dark pink spathe is broadly heart-shaped with slightly overlapping lobes. It also has a yellow reclining spadix.

Three commercially grown cultivars were also examined. The cultivar 'Kaumana' is a high yielding small flowered plant. The flower consists of a dark red open heart-shaped spathe and a reclining spadix. Another dark red cultivar analyzed was 'Kozohara'. Its spathe however, is large and heart-shaped with overlapping lobes. 'Nitta' is an orange cultivar with a broad heart-shaped spathe with overlapping lobes. The origin of these cultivars is obscure.

In addition to the seven cultivars, 36 species representing 10 of the 18 sections described by Engler (1905) were studied with the peroxidase enzyme system (Table 1).

Several different tissues of a few cultivars were used with the peroxidase enzyme system to assess the presence of tissue specificity and to distinguish similarities and differences among tissues by the analysis of zymograms. The tissues analyzed included mature and immature leaves, petiole, peduncle, spathe, spadix and roots. The mature leaf tissue was chosen for all other studies.

Table 1. Anthurium species examined.

Species	Section
A. amnicola	Porphyrochitonium
A. andraeanum	Calomystrium
A. antioquiense	Porphyrochitonium
A. antrophyoides	undetermined
A. armeniense	Calomystrium
A. bakeri	Porphyrochitonium
A. bicollectivum	Porphyrochitonium
A. concinnatum	Belolonchium
A. crassiradicans	Porphyrochitonium
A. crystallinum	Cardiolonchium
A. cuspidatum	Pachyneurium
A. formosum	Calomystrium
A. fiedrichsthalii	Porphyrochitonium
A. gracile	Leptanthurium
A. hacumense	Porphyrochitonium
A. kamemotoanum	Calomystrium
A. lentii	Digitinervium
A. lindenianum	Calomystrium
A. longipetalum	undetermined
A. magnificum	Cardiolonchium
A. nymphaeifolium	Calomystrium
A. paludosum	Porphyrochitonium
A. pentaphyllum var. bombacifolium	Dactylophyllium
A. pentaphyllum var. digitatum	Dactylophyllium
A. radicans	Chamaerepium
A. ravenii	Calomystrium
A. roraimense	Calomystrium
A. roseospadix	Calomystrium
A. scandens	Tetraspermium
A. superbum	undetermined
A. supianum	Belolonchium
A. trinerve	Tetraspermium
A. watermaliense	Pachyneurium
A. warocqueanum	Cardiolonchium
A. wendlingeri	Porphyrochitonium

3.2 Enzyme Extraction

After removal from the plant, samples were prepared for extraction by thoroughly washing in cold water and blotting dry. Samples were either used immediately or stored at 4°C for not longer than 24 hours. Three extraction methods evaluated were:

1) Maceration of tissue with a mortar and pestle in 0.2 M phosphate buffer at pH 7.0.
2) Tissue frozen in liquid nitrogen (LN) and then ground in a LN cooled mortar and pestle, 0.2 M phosphate buffer was then added to make a coarse slurry.
3) Tissue frozen in LN and ground in a LN cooled mortar and pestle. An extraction buffer modified from one described by M. Witter (personal communication) was added to make a coarse slurry. This buffer contained 0.05 M Tris, 0.20 M sucrose, 0.002 M EDTA, 0.01 M ascorbate, 0.006 M sodium bisulfite, 0.012 M diethyl dithiocarbamic acid, 0.02% beta-mercaptoethanol and polyvinylpolypyrrolidone (PVPP). The pH of this buffer was adjusted to 7.3 before adding the mercaptoethanol and PVPP.

Sample extracts for the first two extraction methods were absorbed onto Beckman paper wicks. Lens paper was placed between the wick and the macerate to avoid uptake of

fibrous residue upon the wicks. The wicks were either loaded immediately into the gel or stored at -10°C for later use.

In the third extraction method, the macerate was centrifuged at 32,000 g for 20 minutes. The clear supernatant was collected. The supernatants were either used immediately or frozen in liquid nitrogen and stored at -10°C in vials. As in the two previous methods Beckman paper wicks were used to absorb the supernatant.

3.3 Gel Preparation

Two types of gels were prepared for horizontal electrophoresis. Six percent polyacrylamide, used to evaluate the peroxidase system and 12% starch for all other enzyme systems surveyed.

3.3.1 Polyacrylamide

Following methods described by Brewbaker et al. (1968), polyacrylamide gels were prepared by dissolving 6 grams of gelling agent (Cyanogum-41, Sigma Chemical Co.) in 90 ml of 0.05 M Tris - 0.008 M citrate and 10 ml of 0.03 M lithium hydroxide - 0.19 M boric acid. 1 ml of 10% ammonium persulfate and 0.2 ml of N,N,N',N'-Tetramethylethylene-diamine (TEMED) was then added and this mixture was immediately poured into a 18 cm x 20 cm x 0.3 cm tray, covered with a glass plate and left to harden.

3.3.2 Starch

Twelve percent starch gels were prepared by adding 48 grams of hydrolyzed potato starch (Sigma Chemical Co.) to 400 ml of gel buffer in a vacuum flask. The buffer consisting of a solution of 1 part 0.9 M Tris + 0.02 M EDTA + 0.5 M Borate (TEB) buffer (pH 8.6) and 19 parts water was used for all enzyme systems except esterase. A 9 part Tris-citrate to 1 part lithium borate buffer mixture was used for esterase. Swirling constantly, the starch mixture was heated to 76°C over a small open flame. The flask was then removed from the heat and evacuated using a faucet aspirator until only large bubbles rose to the surface. The gel was poured immediately into a 18 cm x 20 cm x 0.9 cm tray. Remaining air bubbles, if any, were removed with a transfer pipette. The gel was allowed to cool for 30 minutes at room temperature then covered with plastic wrap and refrigerated (4°C) for at least one hour before use.

3.4 Electrophoresis and Enzyme Staining

Horizontal electrophoresis was conducted following methods modified from Brewbaker *et al.* (1968). The gels were loaded by inserting paper wicks into slots cut about 7 cm from the cathodal end. In the polyacrylamide gels, the fast moving Ponceau's dye was placed on each wick to mark the migration front. 0.03 M lithium hydroxide - 0.19 M borate (LB) buffer (pH 8.1) was used as the electrode

buffer for the esterase and peroxidase systems, while a TEB buffer at a 1:6 dilution was used for all other enzyme systems studied. Starting voltages and associated buffers for the eight enzyme systems surveyed are listed in Table 2. Starch gels were run for 4-5 hours while polyacrylamide gels were run for 6-7 hours. The electrophoretic process was conducted at 4°C.

After gels were run, starch gels were horizontally sliced into three pieces, each about 2 mm thick. Staining procedure for each enzyme system was as follows:

Alcohol dehydrogenase (ADH) 25 ml of 0.2 M Tris-HCl (pH 8.0), 3 ml of 95% ethanol, 2 ml of 1% NAD, 5 ml of 0.2% nitro blue tetrazolium (NBT) and 1 ml of 0.2% phenazine methosulfate (PMS) were mixed together. The gel was incubated in the stain at 37°C in the dark for 2 hours.

Esterase (EST) 10 mg of alpha-napthyl acetate were dissolved in 0.5 ml of acetone, 0.5 ml water and 50 ml of 0.1 M phosphate buffer (pH 7.0). 50 mg of Fast Blue RR salt was then dissolved in this solution. The gel was incubated in the stain at 37°C in the dark for 2 hours.

Glutamate-oxaloacetate transaminase (GOT) 200 mg of aspartic acid, 100 mg alpha-ketoglutaric acid, 5 mg of pyridoxal 5' phosphate buffer (pH 7.0) and 1 ml of 0.1 M magnesium chloride. 20 mg of Black K salt dissolved in 2 ml of acetone was then mixed in. The gel was incubated in the stain at 37°C in the dark for 2 hours.

Table 2. Buffer system and initial power used in electrophoretic process of each enzyme.

Enzyme	Buffer System	Initial Power
Alcohol dehydrogenase	TEB[Z]	30 mA
Esterase	LB[Y]	35 mA
Glutamate-oxaloacetate transaminase	TEB	35 mA
Malate dehydrogenase	TEB	30 mA
Peroxidase	LB	40 mA
Phosphoglucose isomerase	TEB	30 mA
Phosphoglucomutase	TEB	30 mA

[Z]Tris-EDTA-Borate (pH 8.6)
[Y]Lithium Borate (pH 8.1)

Malate dehydrogenase (MDH) 25 ml of 0.1 M Tris-HCl (pH 8.0), 25 ml of 0.5 M DL-malic acid (adjusted with NaOH to pH 7.0), 1 ml of 1% NAD, 1 ml of 1% MTT or thyiazol blue and 0.25 ml of 1% PMS were mixed. The gel was incubated in the stain at 37°C in the dark for 2 hours.

Phosphoglucomutase (PGM) 40 mg of alpha-D-glucose-1-phosphate were dissolved in 35 ml of 0.2 M Tris-HCl (pH 8.0), 10 units of glucose-6-phosphate dehydrogenase, 0.5 ml of 1% NADP, 2 ml of 0.2% NBT, 1 ml of 0.2% PMS and 10 ml of magnesium chloride. The gel was incubated in the stain at 37°C in the dark for 2 hours.

Phosphoglucose isomerase (PGI) 20 mg of fructose-6-phosphate were dissolved in 35 ml of Tris-HCl (pH 8.0), 10 units of glucose-6-phosphate dehydrogenase, 0.5 ml of 1% NADP, 2 ml of 0.2% NBT, 1 ml of 0.2% PMS and 10 ml of magnesium chloride. The gel was incubated in the stain at 37°C in the dark for 2 hours.

Peroxidase (Px) 40 ml of 35% ethanol and 4.5 ml glacial acetic acid were slightly heated to dissolve 250 mg of benzidine dihydrochloride. 3 ml of 3% hydrogen peroxide were placed in the developing tray. The gel was stained for 2 minutes at room temperature in the light then rinsed thoroughly with water.

Stained starch gels were fixed overnight in a solution composed of methanol : glacial acetic acid : water (6:4:1) and stored at 4°C. Polyacrylamide gels were also stored at 4°C.

Band migration was recorded either by the simple measurement of total migration distance, in mm, or an Rf value.

$$Rf = \frac{\text{distance enzyme band migrated}}{\text{distance marker dye migrated}}$$

The staining intensity for the bands were also noted and visually categorized as either light, medium or heavy.

3.5 Tissue Study

In this study, samples of the mature and immature leaf, mature and immature petiole, peduncle, spathe, spadix, root and leaf sheath tissues of three A. andraeanum cultivars, 'Kaumana', 'Kozohara' and 'Marian Seefurth', were stained for peroxidase. The Rf value and staining intensity for each band was recorded. Based on this data, differences and similarities of the tissues between and within cultivars were evaluated.

3.6 Cultivar Study

Seven A. andraeanum cultivars were selected for this study, they included 'Kaumana', 'Kozohara', 'Manoa Mist', 'Marian Seefurth', 'Nitta', 'Paradise Pink' and 'Uniwai'. These seven cultivars were evaluated using the enzyme systems alcohol dehydrogenase, esterase, glutamate-oxaloacetate transaminase, malate dehydrogenase peroxidase, phosphoglucose isomerase and phosphoglucomutase. The

mature leaf tissue was used for all enzyme systems surveyed.

3.7 Taxonomic Study

Polyacrylamide gels of mature leaf samples from 36 Anthurium species (Table 1) were stained for peroxidase. The banding patterns of these species were analyzed to assess the taxonomic significance of peroxidase isozymes in the genus Anthurium. Jaccard's similarity coefficients (Sneath and Sokal, 1973) were calculated for 33 taxa and the taxonomic significance of the peroxidase bands was determined by unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis. Species in which no bands were detected were used in the cluster analysis.

IV. RESULTS AND DISCUSSION

4.1 Evaluation of Different Procedures for Enzyme Extraction

Enzyme extraction conducted by macerating the tissue with a mortar and pestle in a 0.2 M phosphate buffer yielded a brown macerate. Electrophoresis of samples obtained from this extraction method resulted in unsatisfactory zymograms. Generally, light banding with streaking was observed, in a few instances, dark streaking made it difficult to distinguish any bands.

Macerates to which a 0.2 M phosphate buffer was added to tissue ground in liquid nitrogen also yielded a brown macerate. The browning or discoloration, however, was not as intense as in the fist method. The clarity of the zymograms obtained from the electrophoresis of these samples was similar to that of the first method. There appeared to be lighter streaking in the gels, but lighter banding was also observed.

Samples obtained from the third extraction method, in which a modified buffer was used to avoid phenolic degradation, displayed no tissue browning. After centrifugation a clear light yellow supernatant was collected. Zymograms obtained from these samples exhibited

the least amount of streaking and the sharpest bands. This extraction method was employed in all subsequent experiments.

Sample preparation for electrophoresis can be complicated by interactions occurring between proteins and other cell matter such as carbohydrates, phenolic compounds, hydrolytic and oxidative enzymes that are normally compartmentalized *in vivo* but mixed during tissue maceration. In some cases, crude extracts can be used as samples (Torres *et al.*, 1978; Torres and Bergh, 1980) in others, protective measures against the interaction of proteins and phenols must be taken to ensure clear zymograms (Wendel and Parks, 1983).

Poor zymograms resulting from dark streaking seems to be related to tissue browning noted during the grinding process. This browning was probably caused by released phenolics and other degradative compounds. Phenols are known to form complexes with proteins and are readily oxidized to quinones, these in turn oxidize essential groups of proteins or form covalent bonds to proteins (Loomis and Battaile, 1966). A protein-phenol interaction would not be surprising, since *Anthurium*, like many other tropical species, is known to contain a number of phenolic compounds (Williams *et al.*, 1981).

The second method combining the use of liquid nitrogen and a phosphate buffer yielded a light brown macerate. The

liquid nitrogen added prior to maceration should have ceased all metabolic activity impeding the reactions which cause browning. However as the phosphate buffer was added and the tissues began to thaw, oxidation reactions could have begun, causing the browning to be observed.

The use of a modified Witter buffer along with grinding in liquid nitrogen and centrifugation seemed to eliminate tissue browning and provide a clearer zymogram. Wendel (1980) reported a buffer containing reducing agents such as ascorbic acid, mercaptoethanol and metabisulfite and a phenol oxidase inhibitor diethyl dithiocarbamate was effective in enzyme extraction from Camellia japonica, a tannin rich plant. Removal of the phenols was reported in some cases to be more effective than the mere prevention of the oxidative reaction (Wilson and Hancock, 1978). Phenols can be removed by the addition of excessive polyvinylpolypyrrolidone (PVPP) and centrifugation. Excessive PVPP can cause phenols, which usually form complexes with proteins, to instead bind with the PVPP. These phenols can then be removed with the PVPP by centrifugation (Kuhns and Fretz, 1978a).

4.2 Tissue Specificity of Peroxidase Isozymes

To determine tissue specificity for peroxidase, different tissues and different stages of some tissues of the cultivars 'Kaumana', 'Kozohara'and 'Marian Seefurth' were examined.

The cultivar 'Kaumana' showed good banding from all tissues except the immature leaf and the leaf sheath (Figure 1). The peduncle, immature petiole and mature leaf tissues all showed a single broad band with bands from the peduncle and mature leaf having similar Rf values. The band from the leaf tissue seemed to be more intensely stained than the other bands. This may indicate higher activity in the mature leaf. The mature petiole and the spathe had a high band (Rf = 0.59) in common, while a band at Rf = 0.465 was found in only the spathe and root tissues.

The cultivar 'Kozohara' showed good banding from all tissues except the immature petiole tissues (Figure 2). All the tissues showing good banding with the exception of the spathe tissues seemed to have the band at approximately Rf = 0.51 in common. The spathe and leaf sheath tissues had darker staining bands, perhaps indicating higher activity of peroxidase in these tissues.

All tissues except the immature leaf tissue and the immature petiole, which was unavailable, displayed good banding in the cultivar 'Marian Seefurth' (Figure 3). The zymogram of the spathe and mature leaf tissues were very similar, each showing bands at Rf = 0.66, 0.60, and 0.53. The mature petiole and peduncle tissues had a light staining band at Rf = 0.575 in common. The petiole also had a band in common with the root tissue of Rf = 0.52.

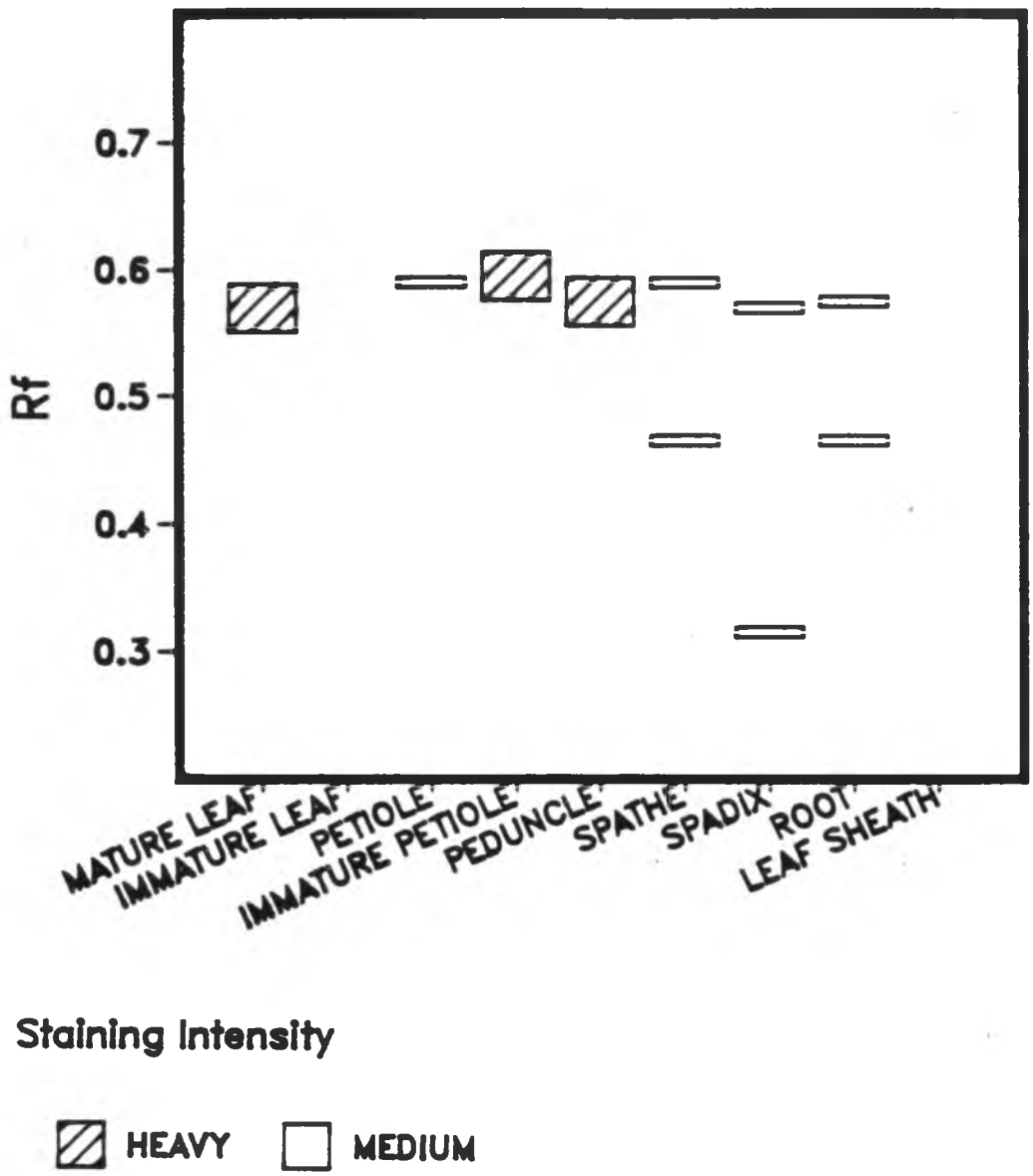


Figure 1. Zymogram of peroxidase isozymes of tissues examined in the cultivar 'Kaumana'.

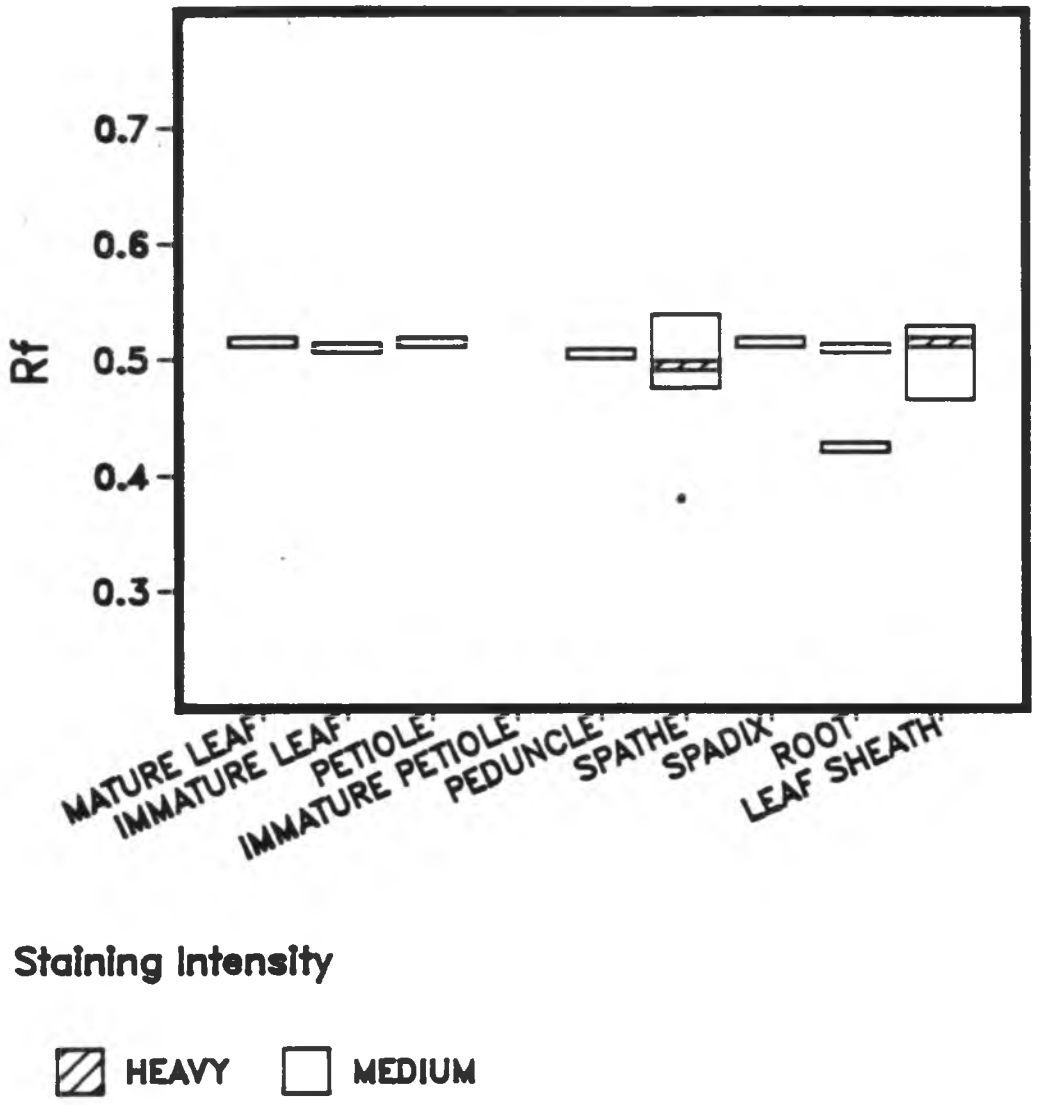


Figure 2. Zymogram of peroxidase isozymes of tissues examined in the cultivar 'Kozohara'.

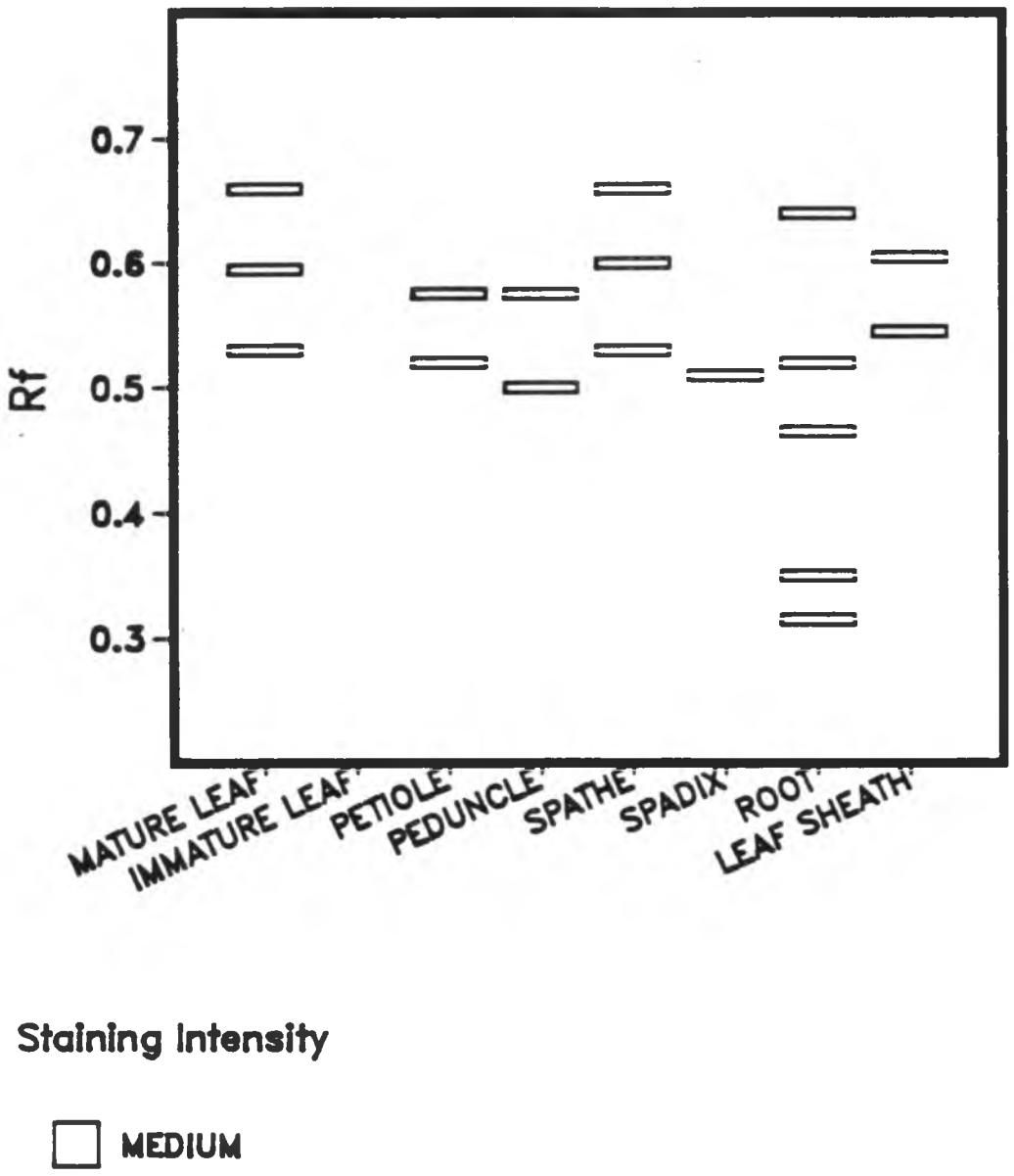


Figure 3. Zymogram of peroxidase isozymes of tissues examined in the cultivar 'Marian Seefurth'.

Peroxidase activity seemed to be most active in the root tissue of this cultivar displaying a total of 5 bands.

The banding patterns between cultivars were compared. The data indicate some specificity among tissues within each cultivar. However, little consistency is observed regarding the similarities and differences of banding patterns among the three cultivars. For example, the zymogram of the spadix tissue of 'Marian Seefurth' can be distinguished from all other tissues of the cultivar. It is difficult however, to distinguish it from the leaf, petiole and spadix tissues of 'Kozohara'.

Although the roles of peroxidase in plants are not completely understood, peroxidase is known to play a vital role in lignification (Harkin and Obst, 1973; Mader and Amberg-Fisher, 1982). It follows then that in many plant species the peroxidase activity in mature tissues are higher than in young immature tissues. The data of this study can neither support nor deny this statement, since no quantitative data were taken. Even on a qualitative basis, based on the number and intensity (ie. heavy, medium or light) of bands the data are somewhat ambiguous. The mature leaf tissue 'Kaumana' and 'Marian Seefurth' seem to have more peroxidase activity than the immature tissue. In 'Kozohara' however, the peroxidase activity in the immature leaf tissues appears to be equivalent to that of the mature leaf tissue. In examination of the petiole tissue,

zymograms pointed to greater activity in the mature tissue of 'Kozohara', whereas in 'Kaumana' the immature tissue seemed to display more activity. Although standardization of tissue fresh weights was attempted, strict control of sample concentrations could not be attained, and variations in total proteins applied to the gel are plausible. These variations may account for some of the ambiguities found in the data.

All tissues tested except the immature leaf and immature petiole, displayed some sort of banding in all three cultivars. The data indicate any one of these tissues which consistently displayed sharp bands can be selected for use in other studies involving peroxidase. The mature leaf tissue was chosen for use in cultivar identification and in genetic and taxonomic studies carried out subsequent to this investigation. Leaf samples were chosen because they were readily available and easier to collect than flower parts or roots. Leaves were also found to be more easily macerated than petioles, peduncles or leaf sheaths and good bands were resolved in all three cultivars examined.

4.3 Cultivar Identification

Bands were resolved in four of the seven enzyme systems surveyed. These included glutamate-oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), peroxidase

(Px) and phosphoglucose isomerase (PGI). Difference in banding intensity was observed in Px. However, since strict control of the total amount of protein applied to each gel was not enforced, quantitative data were not acquired. Although band intensity is a useful descriptive tool, no conclusions can be drawn based on intensity in this study. No staining was observed in gels tested for alcohol dehydrogenase (ADH) and esterase (EST). In the esterase gels however, unresolvable red areas were observed after staining. MacDonald (1974) reported EST bands as blue-black in color. The cause of the red staining is still under investigation. In gels stained for phosphoglucomutase (PGM), some staining was observed, however, bands were not resolved.

In PGI, MDH and GOT only one banding pattern was observed in each system. PGI had one violet-blue band at 5 mm. Band presence was noted in the cultivars, 'Kaumana', 'Kozohara', 'Marian Seefurth' and 'Uniwai'. In 'Nitta', 'Paradise Pink' and 'Manoa Mist' no staining was observed (Figure 4). MDH and GOT each had a banding pattern with two bands at 20 mm and 25 mm, and 3 mm and 20 mm respectively. Violet-blue MDH bands were present in 'Kozohara', 'Nitta' and 'Uniwai' but absent in the four other cultivars (Figure 5). The blue bands of the GOT system appeared to be variable within cultivars. For example, in one zymogram, the cultivars 'Paradise Pink' and

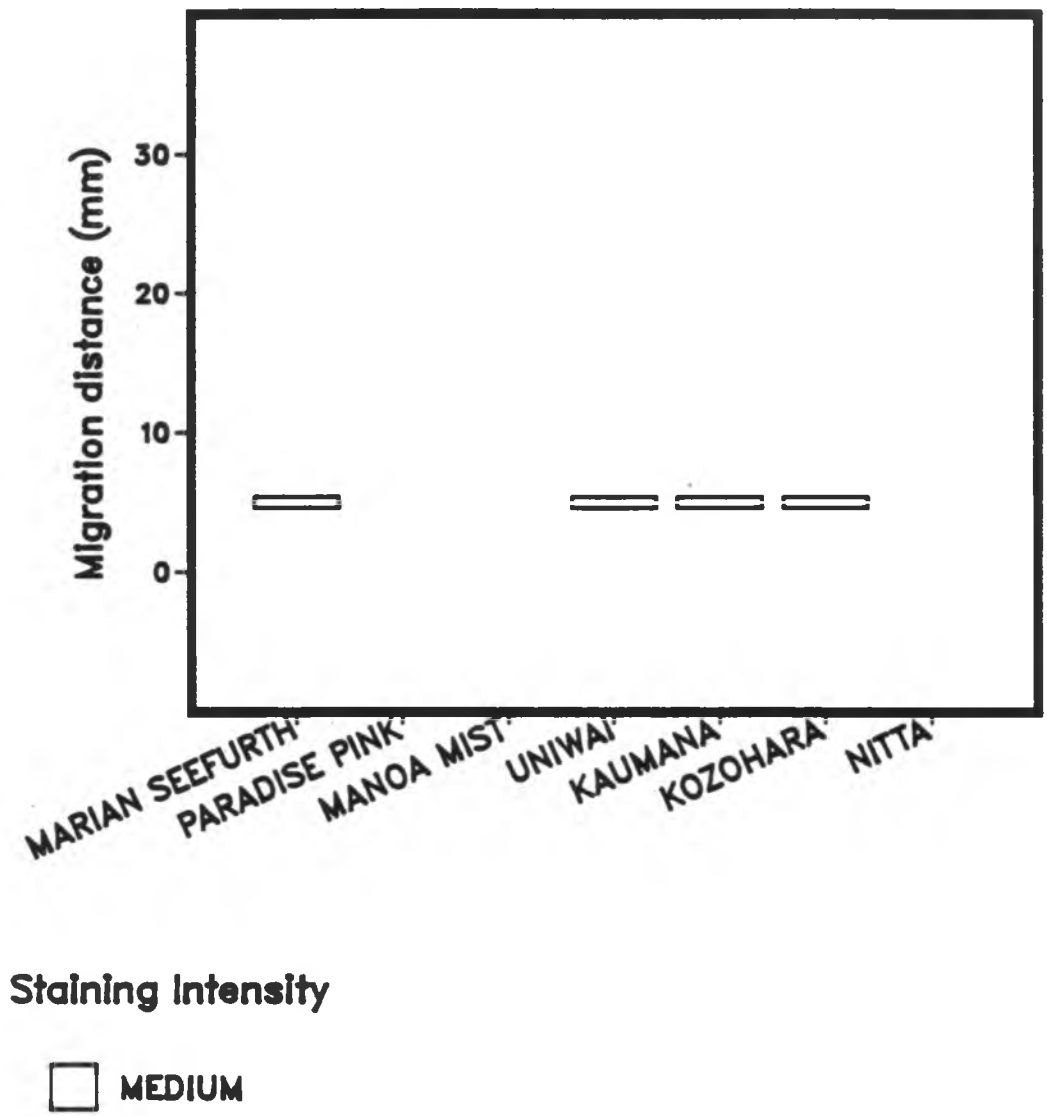


Figure 4. Zymogram of phosphoglucose isomerase isozymes found in mature leaf tissue of 7 anthurium cultivars.

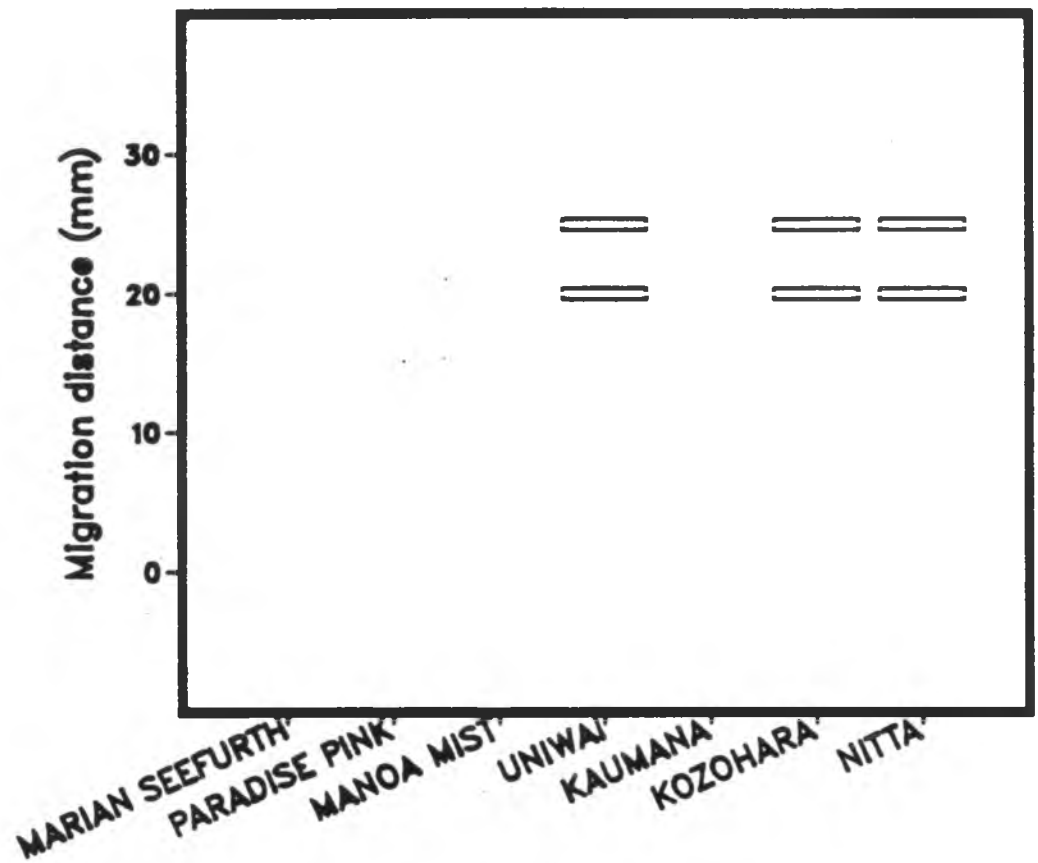


Figure 5. Zymogram of malate dehydrogenase isozymes found in mature leaf tissue of 7 anthurium cultivars.

'Manoa Mist' showed banding and in another zymogram banding was absent. Dark maroon background staining was also observed in GOT gels.

Five distinct banding patterns were resolved in Px (Figure 6). 'Marian Seefurth' and 'Paradise Pink' had similar triple banded patterns, while 'Manoa Mist' and 'Uniwai' each had a broad band around Rf = 0.54. 'Kaumana' also displayed one broad band, but it was located at a higher position, approximately Rf = 0.57. 'Nitta' also exhibited a broad band in the general area of these three cultivars and its very heavy staining along with a band at Rf = 0.305 made it distinguishable from the other cultivars.

Poor banding resolution observed in PGM, EST and ADH can be but is not necessarily, an indication of the absence of enzymes. The results are more likely an indication of incompatibility between these particular enzyme systems and the investigative procedure. There have been many techniques developed with no single one being effective for all enzymes or for all species. Perhaps the use of different extraction methods or buffer systems with different ionic strengths and pHs could elucidate bands from these systems.

Employment of previously described electrophoretic techniques revealed, that out of the seven systems surveyed, Px was the most useful for cultivar separation.

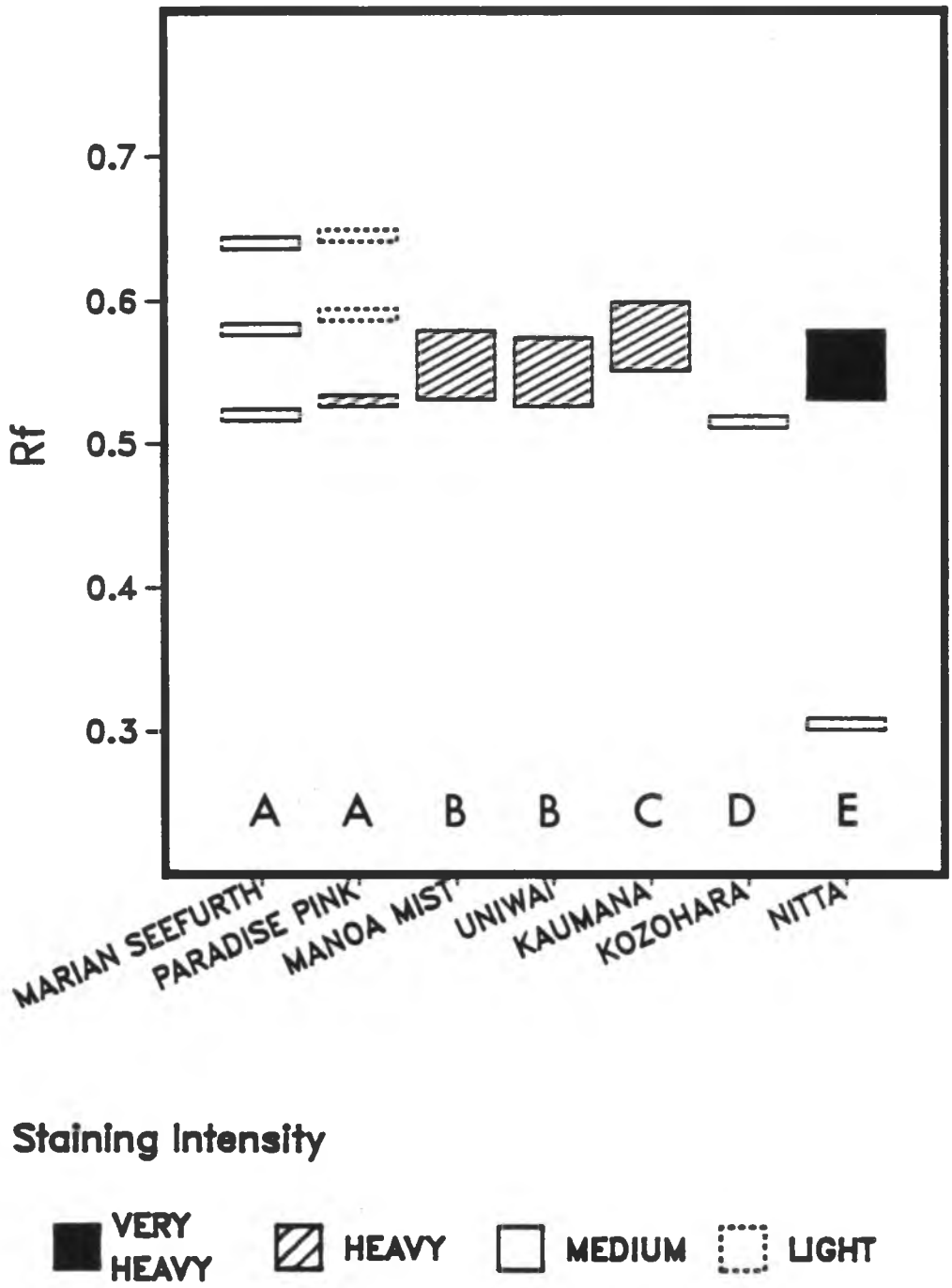


Figure 6. Zymogram of peroxidase isozymes found in mature leaf tissue of 7 anthurium cultivars.

Px was also found to be the most stable. It could be stored frozen for weeks with no noticeable effect on intensity or mobility. As a result, samples could be prepared on various occasions and stored for later use. The other systems however were less stable and fresh samples were used whenever possible. Due to short storage life compounded by time constraints and plant material availability, fewer gels were run in these systems.

The variability noted in GOT can possibly be accounted for by the instability of the system. The variability might also be explained by variations in sample concentration. Although regulation of the amount of tissue used was attempted, the total amount of protein applied to each gel probably was not adequately controlled. Conceivably, light staining bands could be hidden by the dark maroon background.

The peroxidase data suggest slight differences between the Rf values of 'Marian Seefurth' and 'Paradise Pink', and 'Manoa Mist' and 'Uniwai'. The pairs however, were not found to be significantly different. Also, in a combined sample analysis of 'Marian Seefurth' and 'Paradise Pink', the bands appeared to overlap, as the characteristic triple banded pattern was observed. It is therefore believed that the differences are probably due to minor variations in buffer molarities or pH, and rounding error during data compilation.

Genetic similarities seem to be reflected in the Px zymograms. 'Marian Seefurth' and its offspring, 'Paradise Pink'. have very similar zymograms. 'Manoa Mist' also has a similar band pattern to 'Uniwai', one of its parents. Px has been used in genetic studies of various species such as avocado (Torres and Bergh, 1980), corn (Nagai, 1981) and petunia (Berg et al., 1984). The potential use of Px in genetic studies of anthuriums seem to be indicated by the data. It is interesting to note that the white cultivar 'Manoa Mist' has a pattern more similar to its white parent 'Uniwai' than 'Marian Seefurth' its pink parent, and the pink cultivar 'Paradise Pink' is similar to 'Marian Seefurth'. The correlation between spathe color and Px however, was probably more coincidental than genetically linked since little collaborative evidence has been recorded.

The results of this study are preliminary, and therefore it is difficult to draw definite conclusions. Based on Px alone however, 'Kaumana', 'Kozohara' and 'Nitta' appear to be identifiable from the other cultivars. The combined results of Px, MDH and PGI indicated all seven cultivars can be characterized by these systems (Table 3). Thus, isozyme analysis can be a useful tool for distinguishing anthurium cultivars.

TABLE 3. Types of zymograms found in 7 anthurium cultivars for 3 enzymes tested.

Cultivar	Peroxidase*	Malate dehydrogenase	Phosphoglucose isomerase
Kaumana	C	-	+
Kozohara	D	+	+
Nitta	E	+	-
Marian Seefurth	A	-	+
Manoa Mist	B	-	-
Paradise Pink	A	-	-
Uniwai	B	+	+

*A-E represent different peroxidase banding patterns (see Figure 6)
+ indicates presence of band(s)
- indicates absence of band(s)

4.4 Peroxidase as a Taxonomic Aid

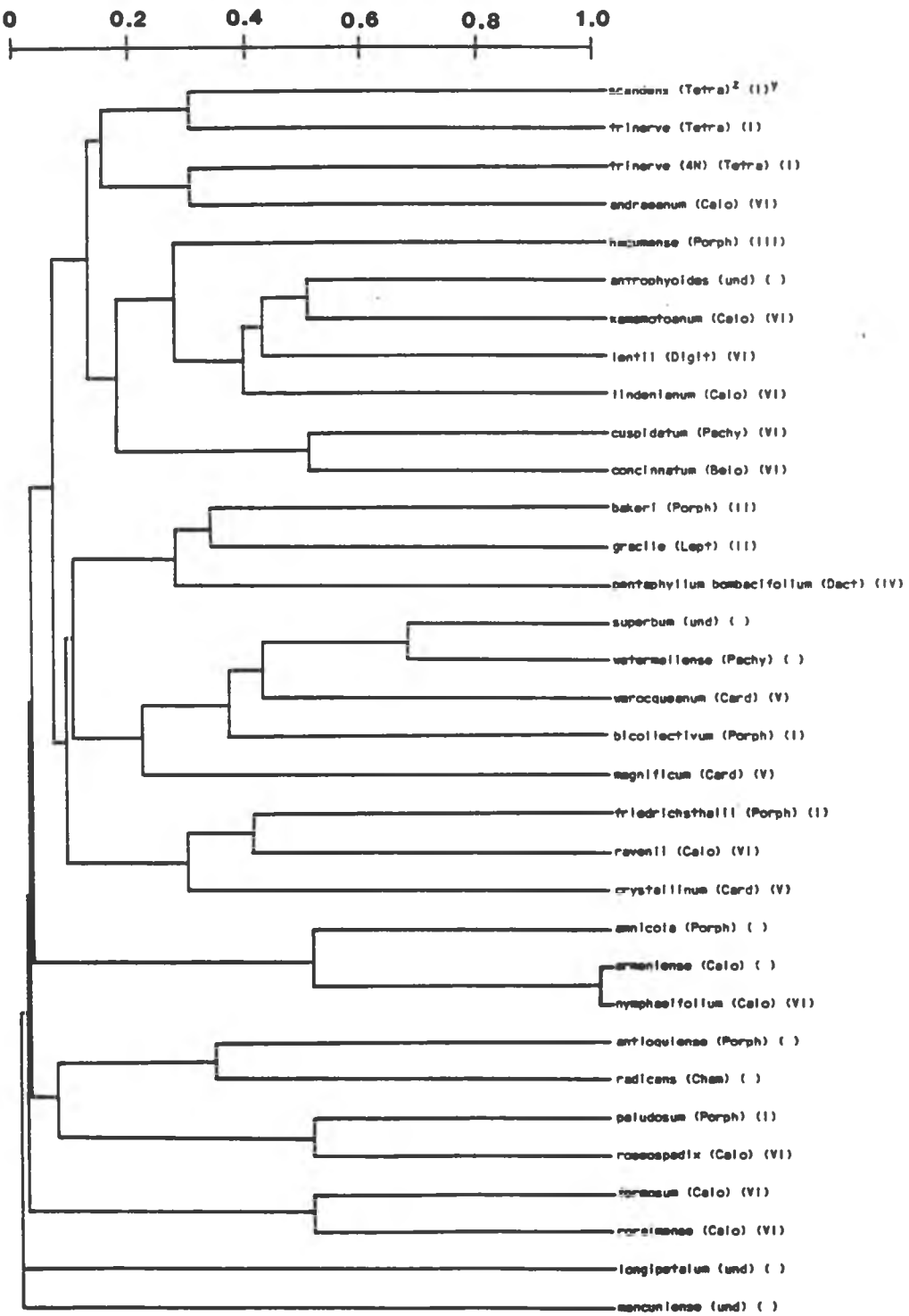
A total of 32 peroxidase positions were recognized for 36 Anthurium species (Figures 8-16). The taxonomic significance of these bands was determined by cluster analysis and the data matrix of Jaccard's similarity coefficients (Sneath and Sokal, 1973) calculated for 33 taxa is shown in Table 4.

The dendrogram (Figure 7) as a whole shows obscure species relationships. However, some component parts of the diagram indicate close species relationships which concur somewhat with taxonomic classifications.

In the top part of the dendrogram, the analysis shows that the species A. scandens and A. trinerve are closely related. This close relationship can be supported by Engler's classification based on plant morphology. According to Engler (1905), both of these species belong to the section Tetraspermium. Sheffer and Kamemoto (1976b) also placed both species into the same group, Group I. Figure 15 shows the strong similarities between the banding patterns of A. scandens and A. trinerve. It is also interesting to note that the two top bands in A. trinerve (4N) are faster moving than the two top bands of the diploid A. trinerve or A. scandens. The dissimilarity was also noted in the cluster analysis which initially grouped A. trinerve (4N) with A. andraeanum. The analysis however,

Table 4. A matrix of Jaccard's similarity measure for 33 anthurium species.

SPECIES	SPECIES																																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1 (scandens)	---	0.250	0.111	0.125	0.200	0.000	0.000	0.000	0.111	0.000	0.125	0.154	0.083	0.125	0.000	0.100	0.222	0.000	0.000	0.000	0.250	0.111	0.222	0.111	0.000	0.000	0.231	0.222	0.222	0.091	0.000	0.275	0.300
2 (trinerve)		---	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.143	0.143	0.000	0.000	0.000	0.000	0.167	0.143	0.000	0.000	0.000	0.182	0.143	0.143	0.125	0.125	0.100
4 (bakeri)			---	0.333	0.143	0.167	0.000	0.000	0.000	0.000	0.143	0.000	0.167	0.300	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.143	0.000	0.000	0.143	0.000	0.000	0.077	0.125	0.125	0.000	0.000
5 (gracile)				---	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.250	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.167	0.200	0.000	0.000	0.200	0.000	0.000	0.333
6 (friedrichsthalii)					---	0.000	0.200	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.333	0.000	0.167
7 (hacumense)						---	0.000	0.000	0.000	0.400	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.143	0.333	0.000	0.200	0.000	0.000	0.000	0.000	0.000	0.250	0.200	0.000	0.000	0.000	0.100	0.200
8 (pentaphyllum var. bombacifolium)							---	0.250	0.250	0.000	0.167	0.000	0.000	0.000	0.250	0.000	0.333	0.111	0.000	0.000	0.000	0.200	0.200	0.000	0.000	0.000	0.250	0.250	0.000	0.250	0.000	0.000	0.000
9 (longipetalum)								---	0.000	0.111	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.182	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000	0.100	0.000	0.000	0.000
10 (mancuniense)									---	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
11 (superbum)										---	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
12 (bicollectivum)											---	0.333	0.333	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.500	0.125	0.167	0.000	0.500	0.000	0.667	0.000	0.000	0.000	0.333	0.000	0.250
13 (amnicola)												---	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.333	0.333	0.000	0.500	0.000	0.000	0.000	0.000	0.000
14 (antrophyoides)													---	0.000	0.000	0.000	0.200	0.500	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.250	0.000	0.500	0.000	0.000	0.000
15 (antioquiense)														---	0.333	0.333	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.500	0.125	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000
16 (paludosum)															---	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.000	0.000
17 (ermenlense)																---	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.000
18 (watermalleuse)																	---	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.143	0.000	0.000	0.000	0.000	0.000	0.060
19 (radicans)																		---	0.250	0.250	0.000	0.000	0.000	0.000	0.000	0.250	0.000	0.333	0.111	0.143	0.000	0.333	0.000
20 (warocqueanum)																			---	0.000	0.000	0.000	0.167	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000
21 (crystallinum)																				---	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000
22 (magnificum)																					---	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200
23 (andreanum)																						---	0.000	0.000	0.000	0.125	0.000	0.000	0.167	0.000	0.000	0.000	0.000
24 (hamemofoanum)																							---	0.125	0.125	0.000	0.000	0.143	0.000	0.000	0.286	0.125	0.143
25 (roseospedix)																								---	0.500	0.500	0.000	0.000	0.000	0.000	0.000	0.500	0.000
26 (lladenianum)																									---	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
27 (formosum)																										---	0.333	0.333	0.000	0.000	0.000	0.000	0.000
28 (roraimense)																											---	0.000	0.000	0.000	0.200	0.000	0.500
29 (nymphaeifolium)																												---	0.000	0.000	0.000	0.250	0.000
30 (ravenii)																													---	0.000	0.000	0.000	0.000
31 (cuspidatum)																														---	0.000	0.000	0.000
32 (lentii)																															---	0.500	0.000
33 (concinnatum)																																---	0.333



Z Engler section — Belo = Belolonchium, Calo = Calomystrium, Card = Cardiolonchium, Cham = Chamaerepium, Dact = Dactylophyllium, Digit = Digitinervium, Lept = Leptanthurium, Pachy = Pachyneurium, Porph = Porphyrochitonium, Tetra = Tetraspermium, und = section undetermined.
Y Sheffer and Kamemoto group — roman numeral represents Group number.

Figure 7. UPGMA cluster analysis of 33 anthurium species using Jaccard's similarity index on peroxidase isozyme data.

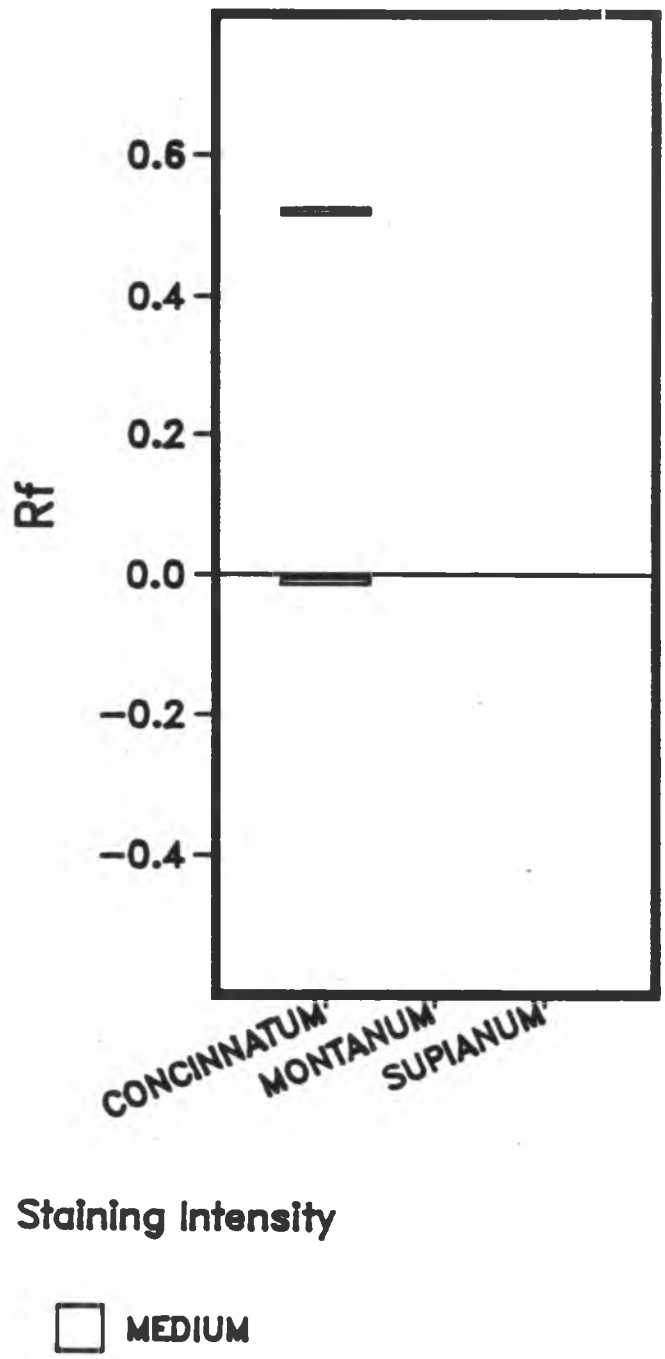


Figure 8. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in the section Belolonchium.

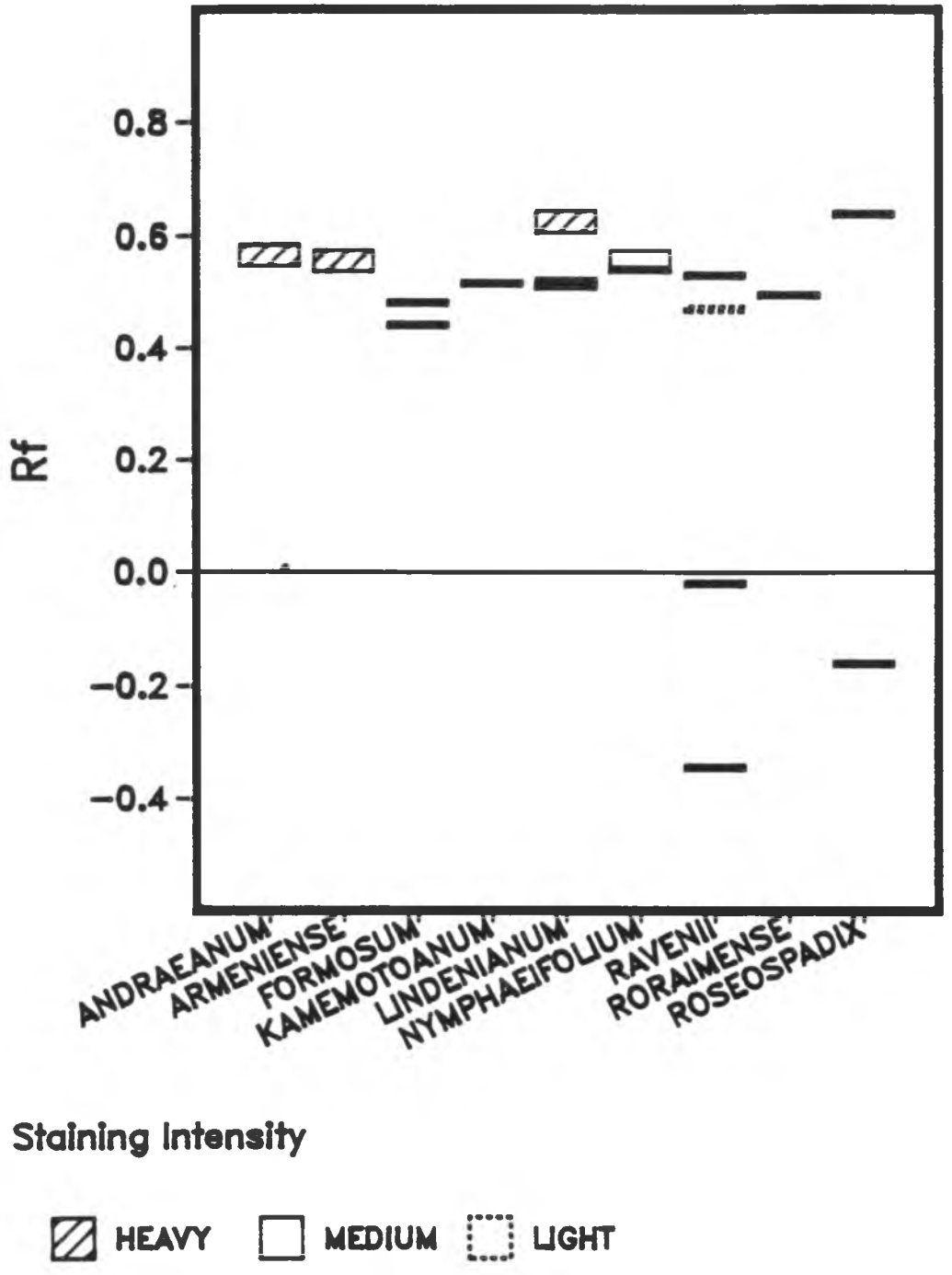


Figure 9. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in the section Calomystrium.

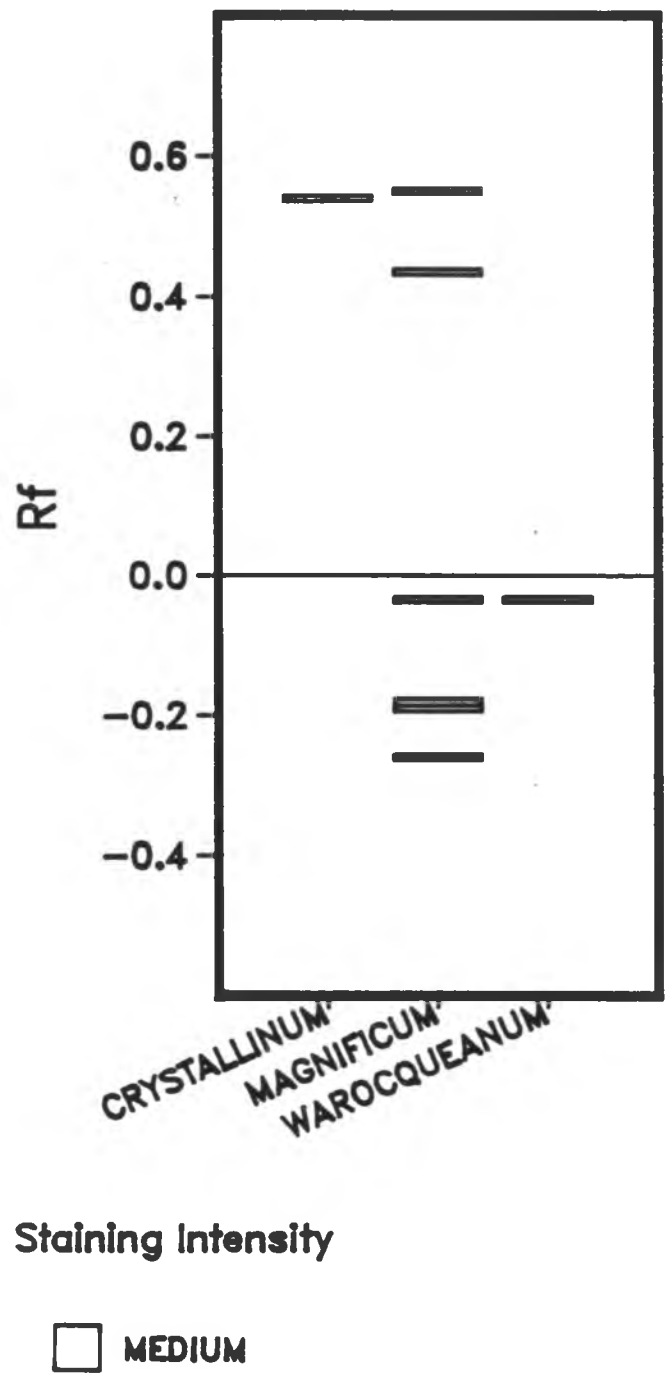


Figure 10. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in the section Cardiolonchium.

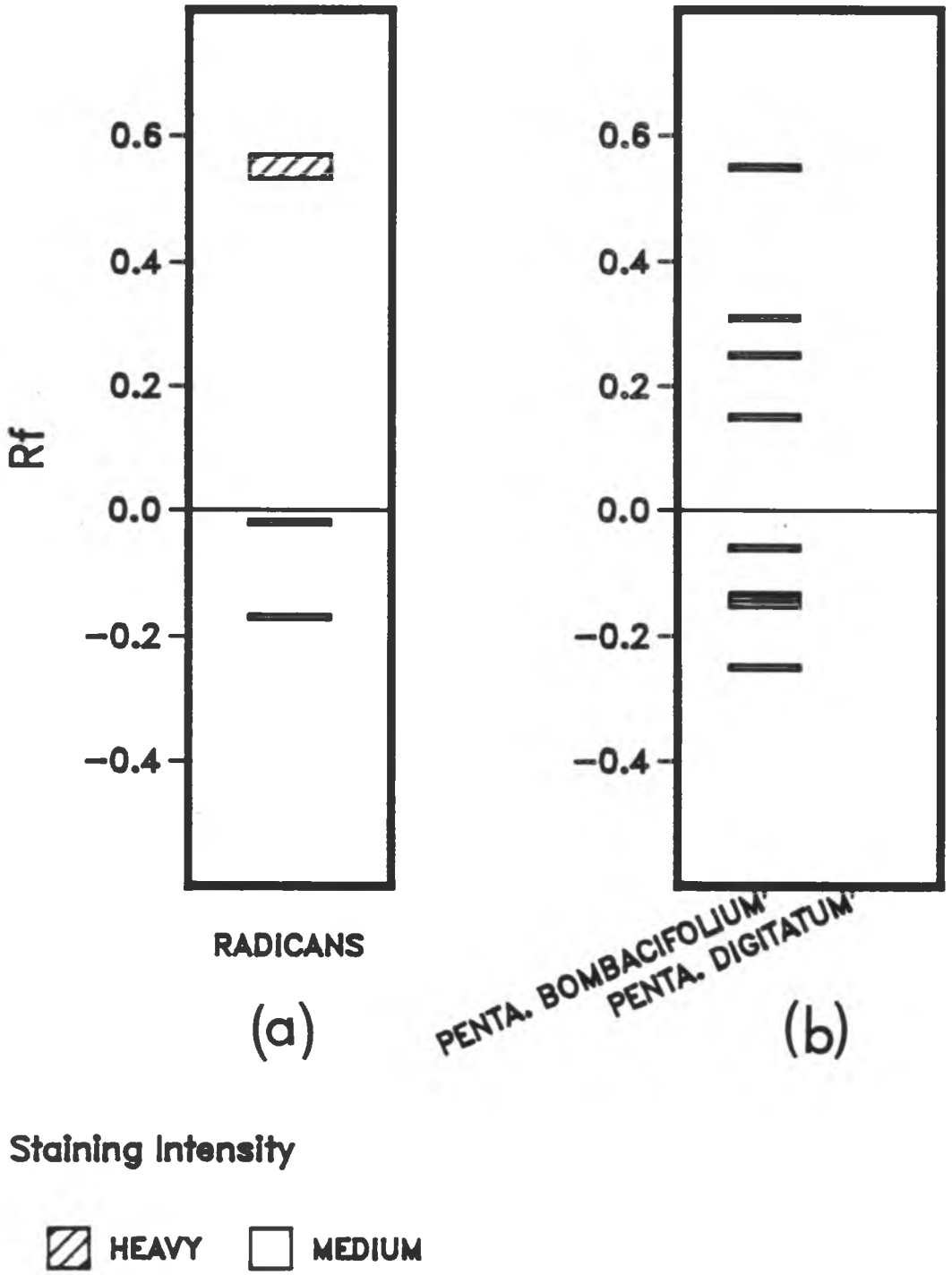


Figure 11. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in the sections (a) Chamaerepium, (b) Dactylophyllium.

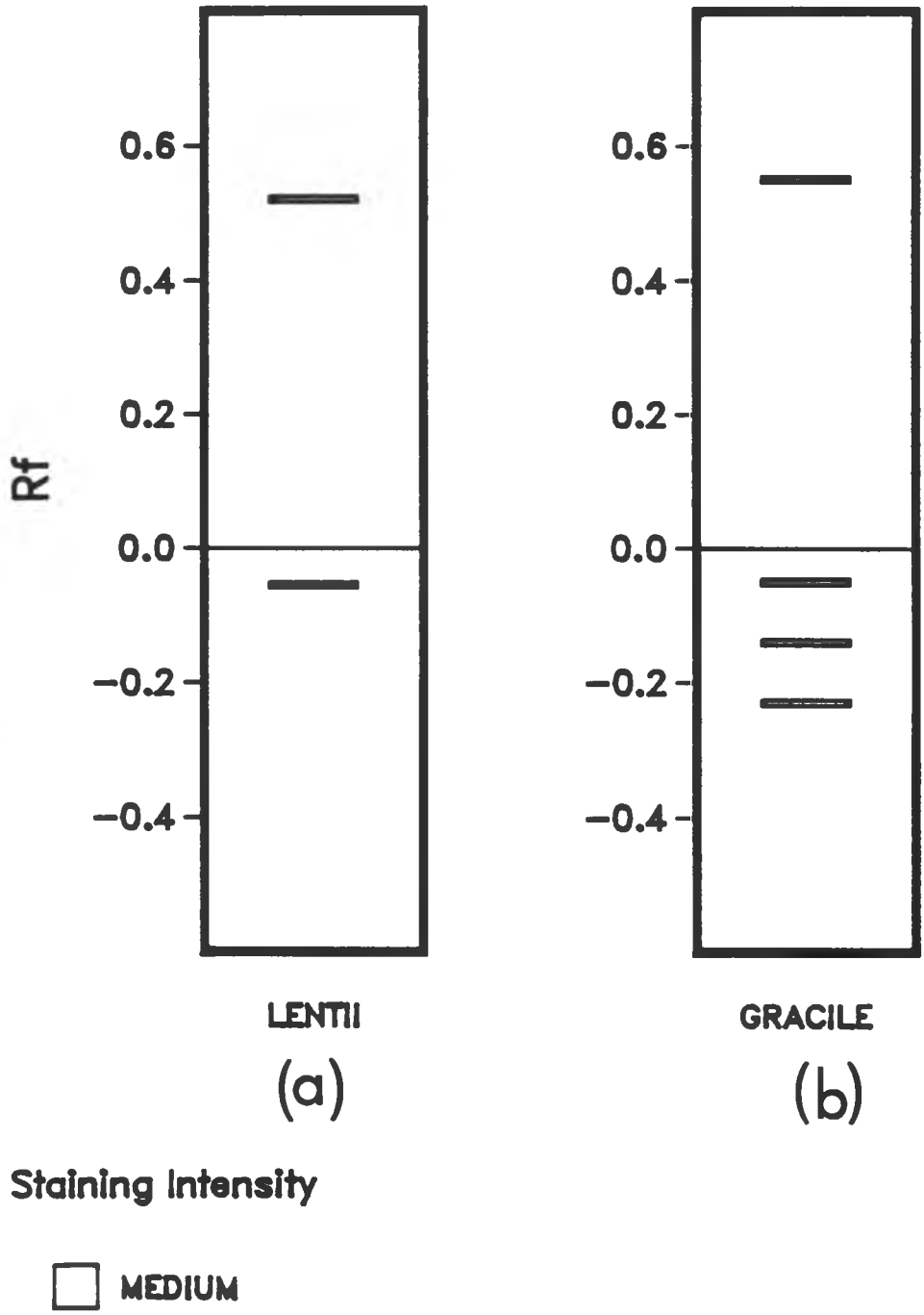


Figure 12. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in the sections (a) Digitinervium, (b) Leptanthurium.

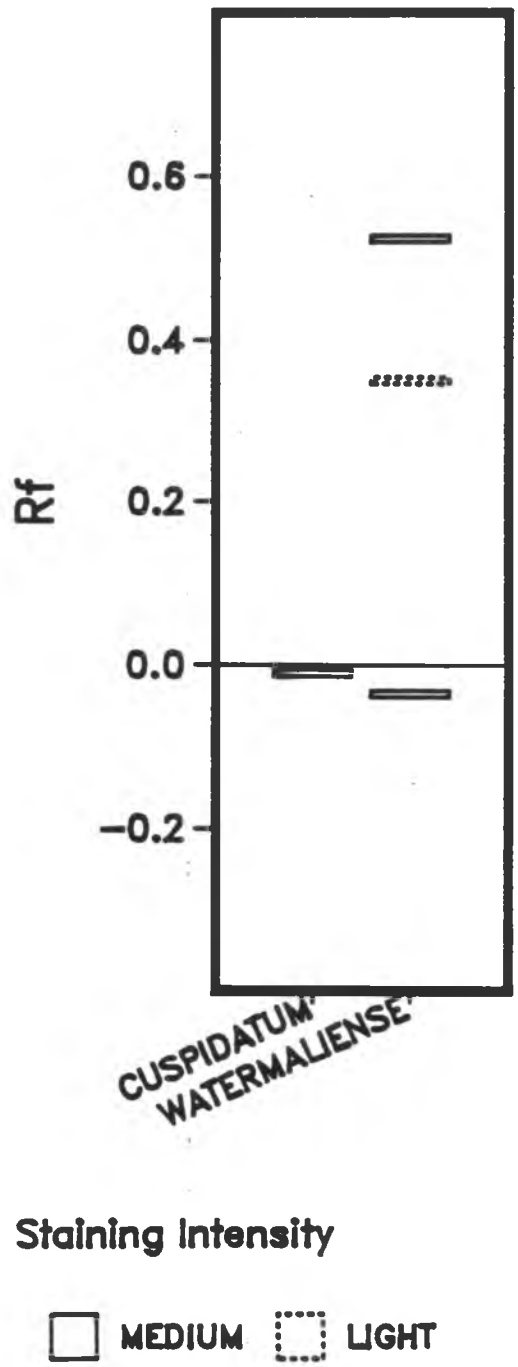


Figure 13. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in the section Pachyneurium.

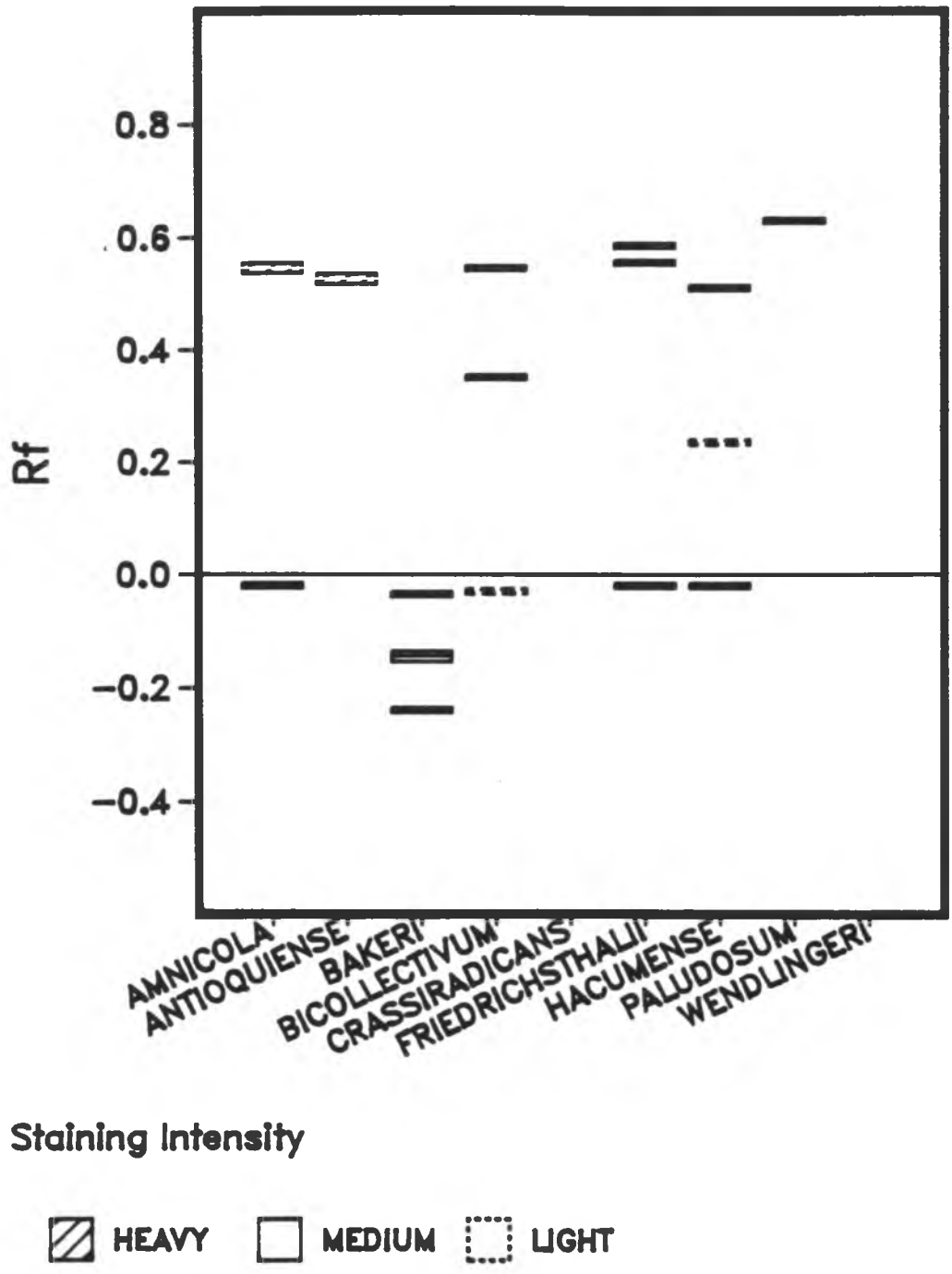


Figure 14. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in the section Porphyrochitonium.

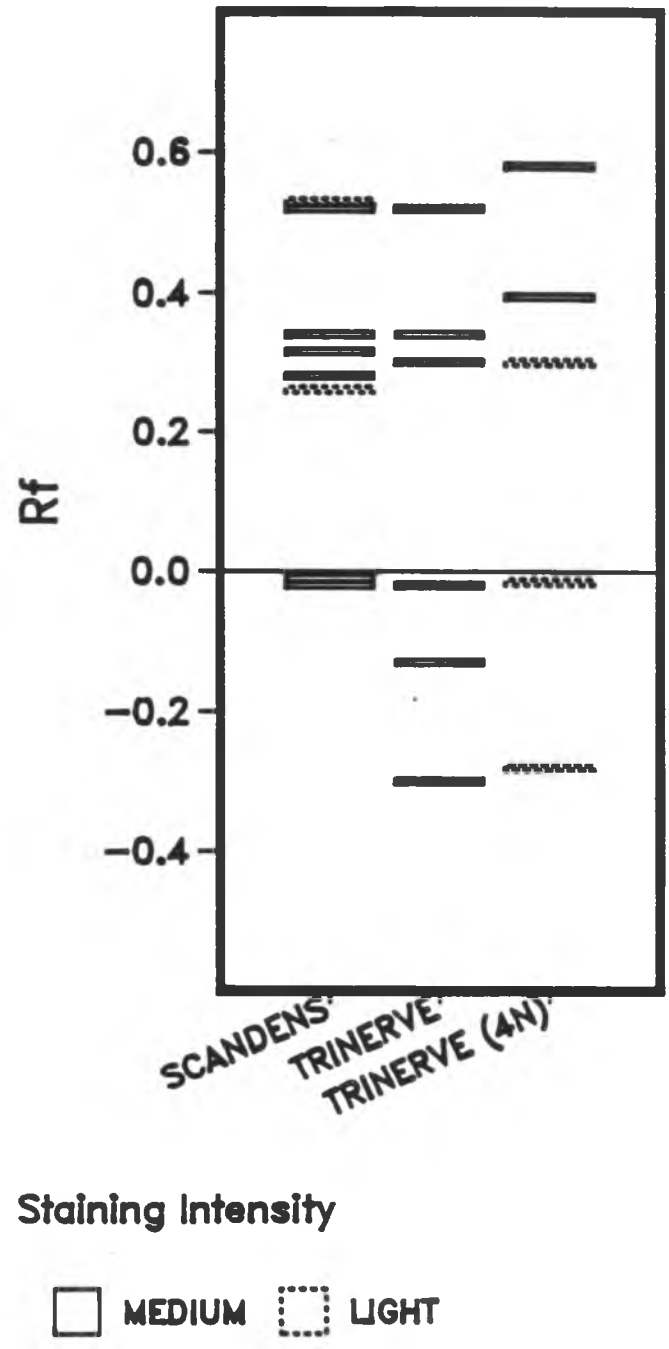


Figure 15. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in the section Tetraspermium.

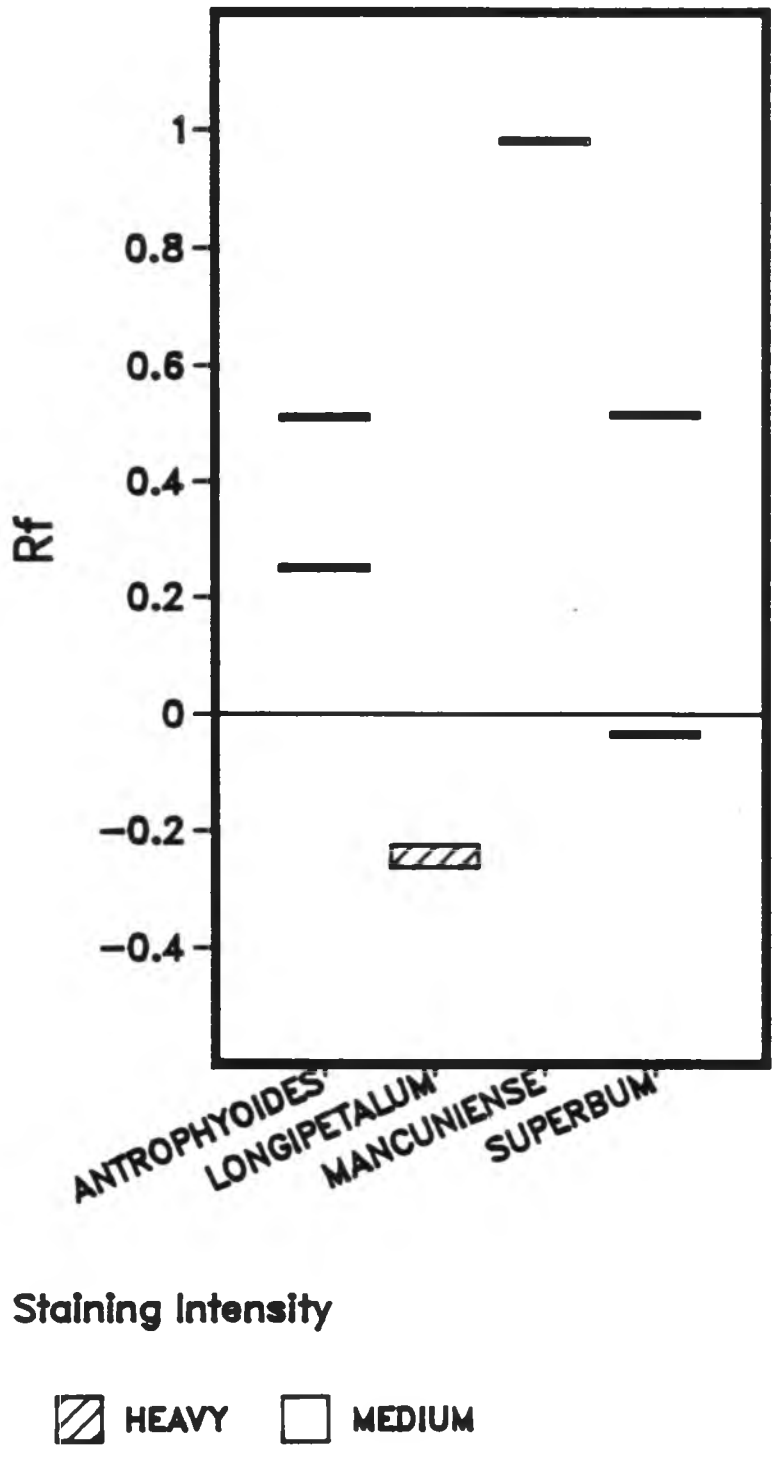


Figure 16. Zymogram of peroxidase isozymes of mature leaf tissue found in the species for which sections have not yet been determined.

also indicated that the group of A. trinerve (4N) and A. andraeanum is more closely related to the group of A. scandens and A. trinerve than any other group.

The cluster analysis also grouped together the species A. antrophyoides, A. kamemotoanum, A. lentii, A. lindenianum, A. hacumense, A. cuspidatum and A. concinnatum. Five of the seven species were morphologically grouped together by Sheffer and Kamemoto. Although Sheffer and Kamemoto's classification differs from Engler's, in their groupings they found that the crossability of plants within any one group was higher than between different groups. They therefore concluded that plants within groups were probably closely related.

Two other species which seem to be closely related are A. bakeri and A. gracile (Figure 18). Sheffer and Kamemoto placed both of these species in Group II, Engler's classification however, placed them into two different sections, Porphyrochitonium and Leptanthurium respectively. The cluster analysis tended to follow the Sheffer and Kamemoto classification as the analysis indicated that of the 33 taxa examined, A. bakeri is most closely related to A. gracile.

The analysis also found the species A. armeniense to be most closely related to A. nymphaeifolium, and A. formosum to be most closely related to A. roraimense. All four of

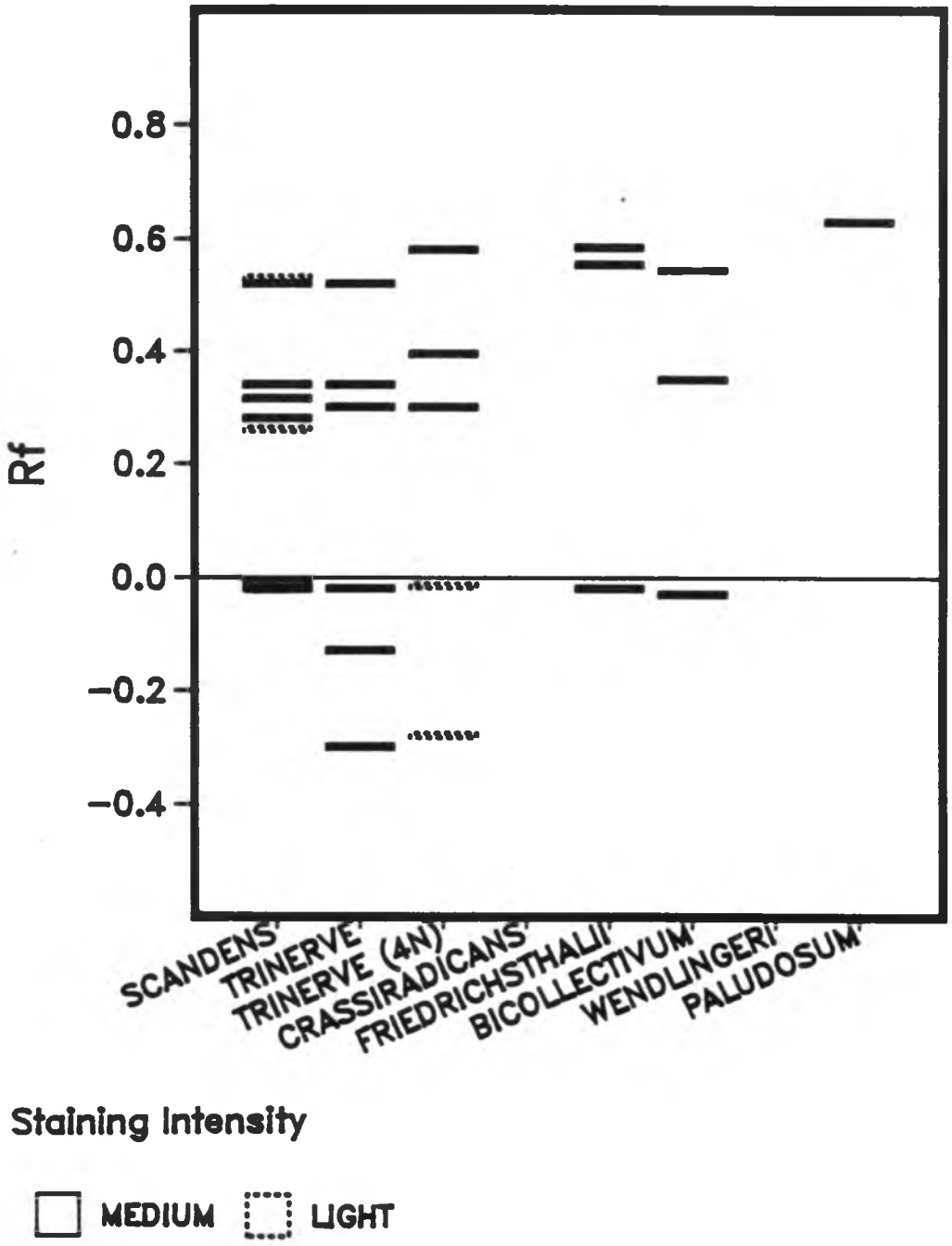


Figure 17. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in Group I.

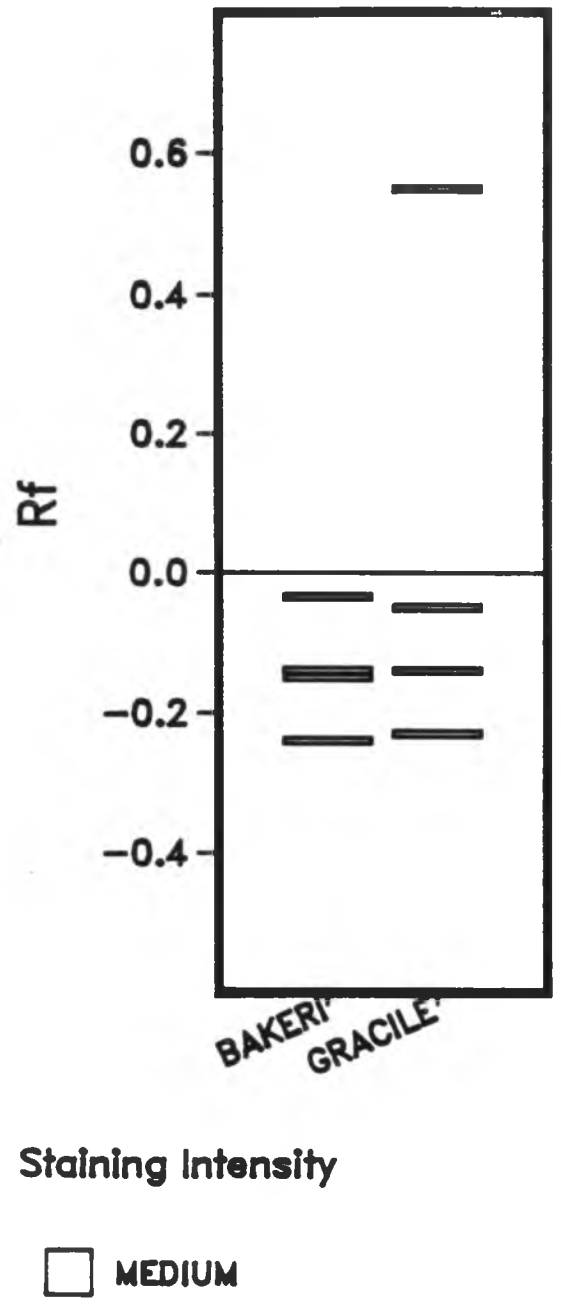


Figure 18. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in Group II.

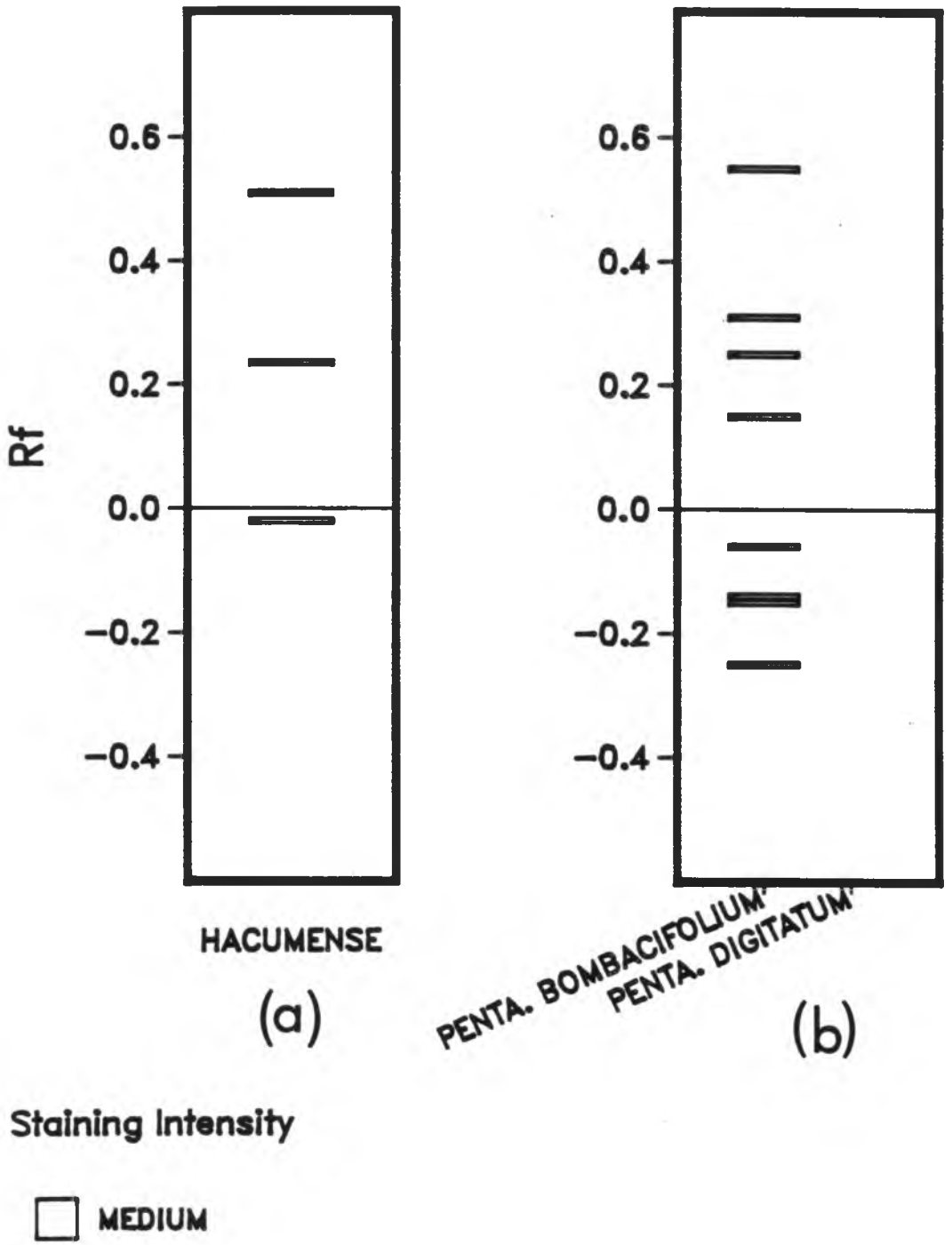


Figure 19. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in (a) Group III, (b) Group IV .

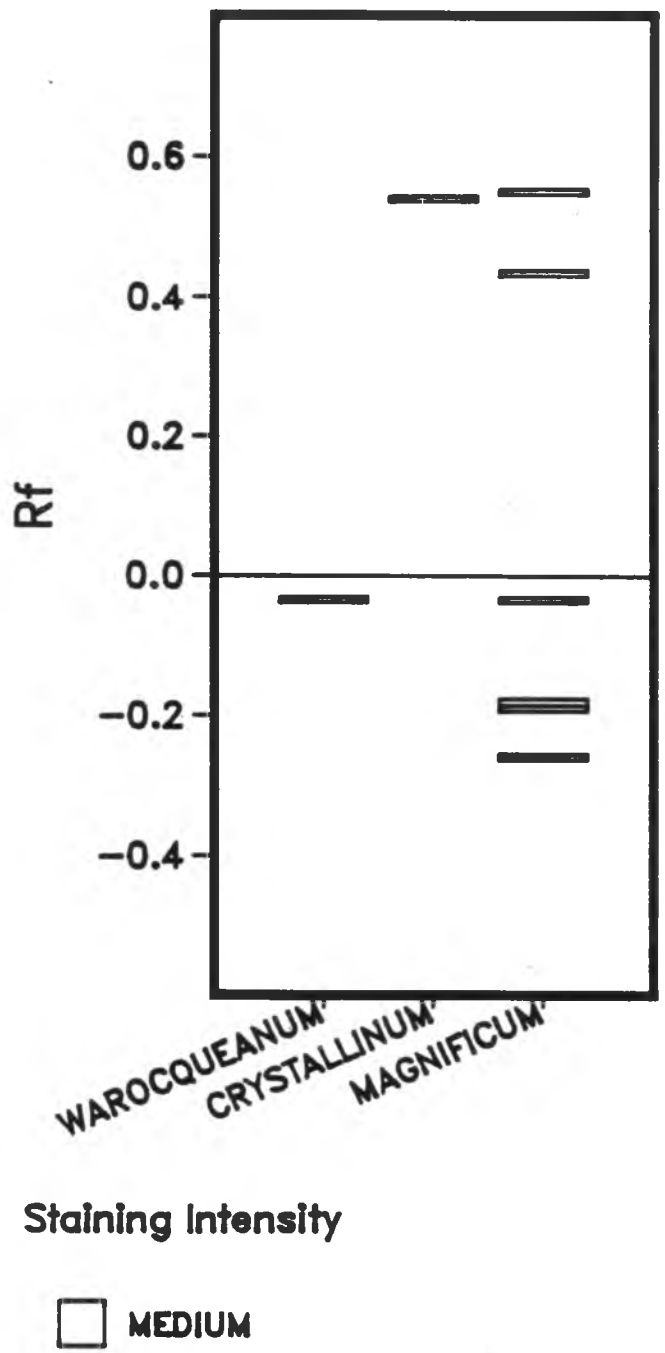


Figure 20. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in Group V.

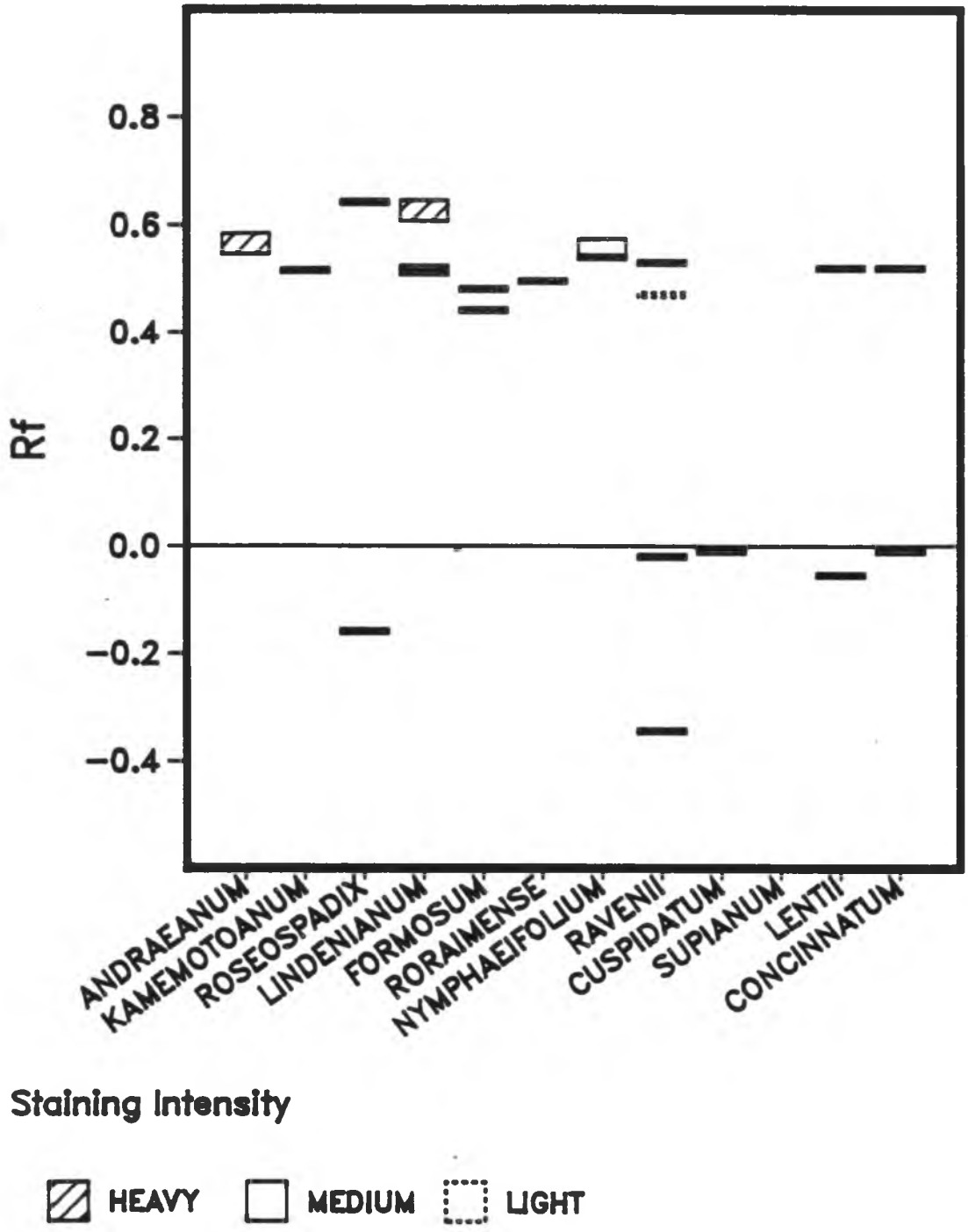


Figure 21. Zymogram of peroxidase isozymes of mature leaf tissue found in the species in Group VI.

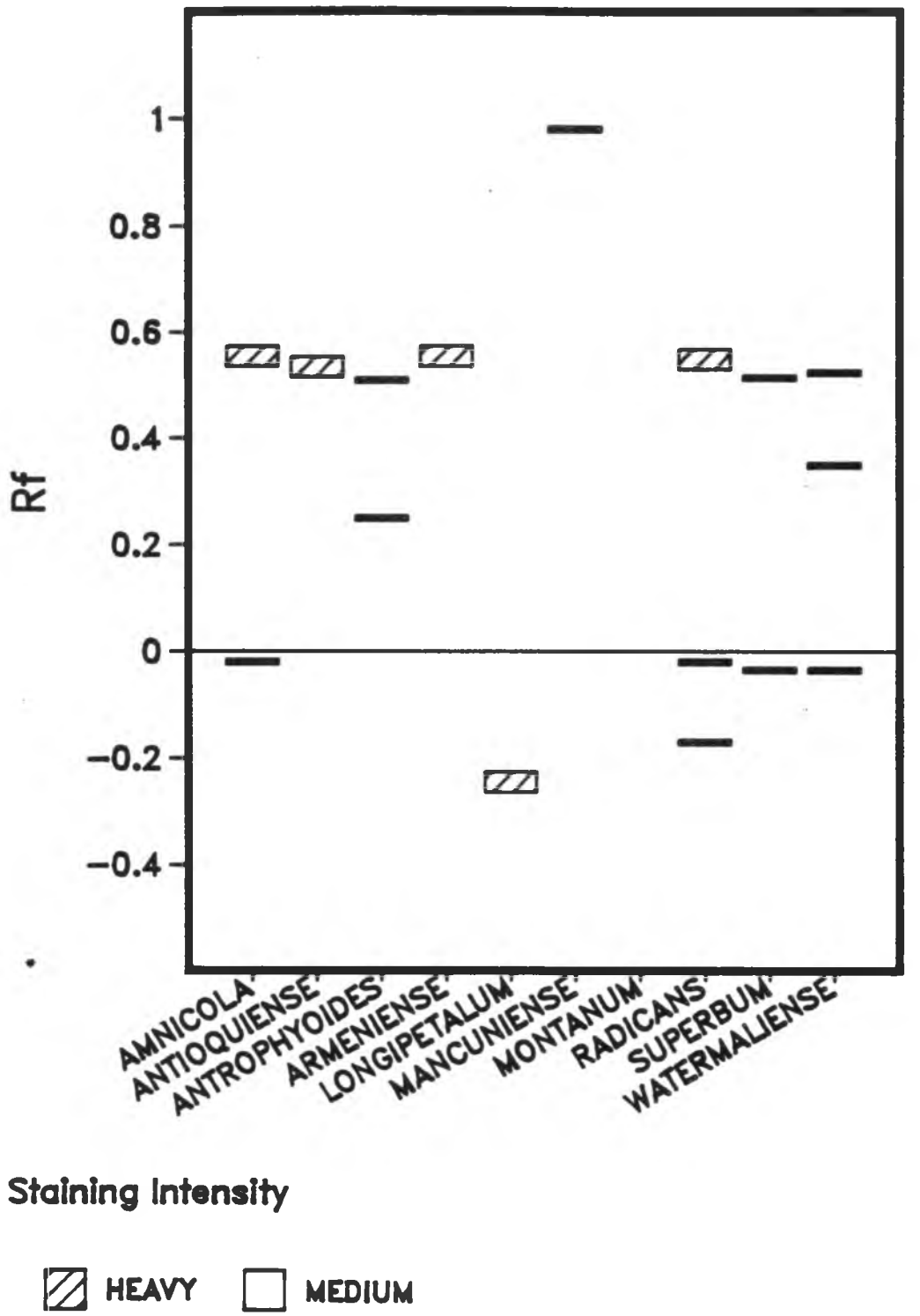


Figure 22. Zymogram of peroxidase isozymes of mature leaf tissue found in the species for which groups have not been determined.

these species were classified in the Engler section Calomystrium. However a close relationship was not found between the two clusters.

Although the Sheffer and Kamemoto classification was based on important Englerian characters, in general the isozyme data tended to follow the Sheffer and Kamemoto classification. Zymograms of the 36 species are shown in Figures 8-16 and 17-22 as classified by Engler and Sheffer and Kamemoto respectively. In three of the six Sheffer and Kamemoto groups, Group I (Figure 17), Group II (Figure 18) and Group VI (Figure 21), most of the species within each group show banding in similar Rf areas. However, the banding patterns of the species within only two Engler sections, Calomystrium (Figure 9) and Tetraspermium (Figure 15) showed some level of consistency. One reason the data more closely follows Sheffer and Kamemoto's classification may be that there are differences in the structure of the two classifications. Engler did not consider the number of seeds per berry whereas Sheffer and Kamemoto did use this character in their classification. Engler also classified the genus into 18 sections, whereas Sheffer and Kamemoto established only six groups. Since only 33 species were examined, some of the Englerian sections were represented by only one or two species. Thus, it was difficult to assess species and section relationships.

It should be noted that species in which no bands were detected were not included in this analysis due to the limitations of the analytic procedure. In this cluster procedure, the taxa were grouped on the basis of "positive" matches, where "positive" is equivalent to band presence and "negative" is equivalent to band absence. In the case of the species with no bands present, there would be no "positive" matches and the cluster analysis would indicate that all these species were not related to any of the other species.

The incongruities observed in the overall dendrogram might have been due to limited data for the large number of taxa involved. However, even with data from only one enzyme system, some isozyme similarities seem to parallel morphologic similarities. Since a character or group of characters will probably be more significant within a limited group (Radford et al., 1974), the analysis might have been more meaningful if less taxa were involved.

The purpose of this study was to determine the significance of peroxidase isozymes for taxonomic purposes and therefore only peroxidase bands were used as characters in this analysis. From this data, it was found that the peroxidase system, used alone did not adequately assess all relationships. However, in a taxonomic study, rarely are isozymes used alone to determine relationships; they are rather considered a single character or a small group of

characters and are used in conjunction with other genetic and morphologic data. The data from this study seem to indicate that isozymes can be considered a useful character to use in relationships. In any case more enzyme systems should be included in the analysis before the role of isozymes in anthurium taxonomy can be ascertained.

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